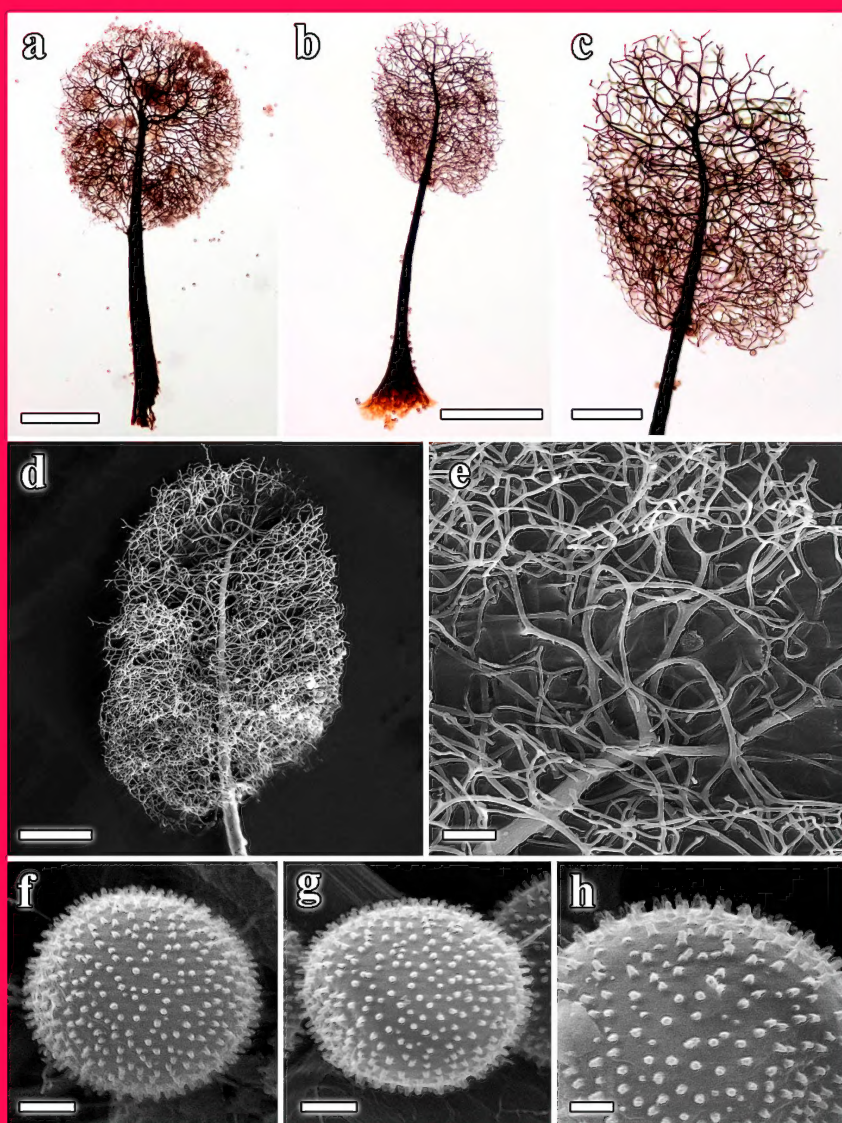


MYCOTAXON

THE INTERNATIONAL JOURNAL OF FUNGAL TAXONOMY & NOMENCLATURE

VOLUME 133 (3)

JULY–SEPTEMBER 2018



Comatricha parvula sp. nov.

(Moreno & al. — FIG. 7, p. 533)

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LORELEI L. NORVELL

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Pacific Northwest Mycology Service

6720 NW Skyline Boulevard

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MYCOTAXON

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The Editors express their appreciation to the following individuals who have, prior to acceptance for publication, reviewed one or more of the papers prepared for this quarter.

Elvira Aguirre-Acosta

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FROM THE EDITOR-IN-CHIEF

PROGRESS! Your Editor is pleased to report that she is closing the gap and dares to dream that our next quarterly issue will be released ‘almost’ on time. Despite our best efforts, we have managed to release the last issue of the year in the year embossed on the cover only three times since 2004, but we plan to pull off that miracle in 2018. Our decision to turn out papers in a timely manner has admittedly resulted in another slim issue, but we anticipate that an increased number of final submissions prompted by our removal of mandatory page charges will increase the pages per issue considerably by this time next year.

THE MYCOTAXON 2018 JULY–SEPTEMBER ISSUE presents 18 research papers by 67 authors (representing 11 countries) expertly reviewed by 35 mycological peers.

Within its brightly colored covers appear one new genus (*Saksenamyces* from India) and eight species new to science representing *Comatricha* from Spain, *Hymenochaete* & *Tulostoma* from Mexico, *Pestalotiopsis* & *Vamsapriya* from China, and *Saksenamyces* from India.

Happily, our current requirements for range extension papers have helped make the reported identifications far more reliable: for over a year we have asked authors to compare their new specimens with type material or type descriptions and provide illustrations of their material for our readers. MYCOTAXON 133(3) covers first reports of *Garnaudia* for China, *Planetella* for Greenland and other smut fungi for Korea, myxomycetes for China and Spain, ascotruffles *Hydnobolites* & *Pachyphlodes* for Turkey, and hypogeous *Alpova* & *Rhizopogon* for Brazil. China continues its beautifully illustrated treatments of lichens through three excellent range extensions covering 21 species representing *Arthonia*, *Bryobilimbia*, *Byssoloma*, *Calenia*, *Catillaria*, *Coenogonium*, *Chroodiscus*, *Mycobilimbia*, *Eremothecella*, *Mycobilimbia*, *Pyrenula*, *Semigyalecta*, and *Toninia*.

Even more convincing are the sequence analyses supporting range extensions (*Cantharocybe* in Thailand, *Mucor irregularis* in South America), new host associations (a new *Puccinia-Berberis* aecial host association in Pakistan), and new taxonomic relationships (a powdery mildew from China transferred to *Cystotheca*) or species (*Pestalotiopsis lijiangensis*, *Tulostoma rufescens*). The combination of traditional morphological and cultural methods supported by recent technological advances ensures the continuing acceptance of taxonomic mycology as a thriving and vital science.

Warm regards,

Lorelei Norvell (*Editor-in-Chief*)

24 October 2018

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***Vamsapriya jinniuensis* sp. nov., and a first record of *Garnaudia elegans* from southern China**

YIN LING¹, HAO-HUA LI², JI-WEN XIA²,
XIU-GUO ZHANG^{2*}A, ZHUANG LI^{2*}B

¹ College of Life Sciences, Inner Mongolia University for Nationalities,
Tongliao, Inner Mongolia 028000, China

² Shandong Provincial Key Laboratory for Biology of Vegetable Diseases and Insect Pests,
College of Plant Protection, Shandong Agricultural University, Taian, 271018, China

*CORRESPONDENCE TO: ^a sdau613@163.com zhxg@sdau.edu.cn ^b liz552@126.com

ABSTRACT —A new species, *Vamsapriya jinniuensis*, is described and illustrated from specimens collected on dead stems in Hainan Province. The fungus is unique in producing catenate, phragmosporous, obclavate conidia on synnematos conidiophores with non-cicatrized, monotretic conidiogenous cells. *Garnaudia elegans* is newly recorded from China.

KEY WORDS —hyphomycetes, taxonomy, Xylariaceae

Introduction

During our ongoing survey of microfungi associated with woody debris in the forests of southern China, two specimens with the morphological characteristics of *Vamsapriya* Gawas & Bhat (Gawas & Bhat 2006) and *Garnaudia* Borowska (Borowska 1977) were collected on dead stems. *Vamsapriya jinniuensis* is described here as a new species, and *Garnaudia elegans* represents a new record for China. *Vamsapriya* currently includes six species: *V. bambusicola* D.Q. Dai & al., *V. breviconidiophora* Jun F. Li & al., *V. camagueyensis* (R.F. Castañeda) R.F. Castañeda & al., *V. indica* Gawas & Bhat, *V. khunkonensis* D.Q. Dai & al., and *V. mahabaleshwarensis* J. Pratibha & Bhat (Gawas & Bhat 2006, Pratibha & Bhat 2008, Dai & al. 2015, Castañeda-Ruiz & al. 2017, Hyde & al. 2017). Specimens are deposited in the

Herbarium of the Department of Plant Pathology, Shandong Agricultural University, Taian, China (HSAUP) and the Mycological Herbarium, Institute of Microbiology, Chinese Academy of Sciences, Beijing, China (HMAS).

Vamsapriya jinniuiensis Yin Ling & X.G. Zhang, sp. nov.

FIG. 1

MYCOBANK MB 826967

Differs from *Vamsapriya indica* by its longer, narrower, obclavate conidia.

TYPE: China, Hainan Province: Jinniuling, on dead stems of an unidentified broadleaf tree, 22 Feb. 2017, H.H. Li (Holotype, HSAUP H10242; isotype, HMAS 245647).

ETYMOLOGY: *jinniuiensis*, in reference to the type locality, Jinniuling.

COLONIES on natural substrate effuse, dark brown to black. Mycelium mostly immersed in the substratum, composed of subhyaline, septate, branched, smooth hyphae. Synnemata erect, rigid, dark brown, composed of compact parallel conidiophores, ≤ 875 μm high, 36.5–103.5 μm diam. CONIDIOPHORES distinct, dark brown, smooth, septate, branched, 4–4.5 μm diam. CONIDIOGENOUS CELLS monotretic, uncitrized, integrated or discrete, terminal, clavate, slightly curved toward the exterior, 12–16 $\times$ 3.8–4.5 μm . CONIDIA dry, catenate, acrogenous, brown, smooth, simple, obclavate, 9–13-euseptate, constricted at the septa, 76–118.5 $\times$ 2.5–5 μm .

COMMENTS – *Vamsapriya* was established by Gawas & Bhat (2006), with *V. indica* as type species, to accommodate conidial fungi with catenate, phragmosporous conidia on synnematous conidiophores with non-citrized, monotretic, conidiogenous cells (Gawas & Bhat 2006). The genus is similar to *Didymobotryum* Sacc. and *Podosporium* Schwein. in having synnematous conidiomata and monotretic conidiogenous cells. However, conidia in *Didymobotryum* are catenate, ellipsoidal to cylindrical, and 1-septate, while in *Podosporium* they are solitary, obclavate, and multiseptate. *Vamsapriya* combines conidial morphology from both genera, bearing catenate, cylindrical to vermiform, multiseptate conidia.

Vamsapriya indica differs from *V. jinniuiensis*, which has shorter, wider, cylindrical conidia with fewer septa (10–80 $\times$ 4–6 μm , 2–12-euseptate; Gawas & Bhat 2006). The other five *Vamsapriya* species also all differ from *V. jinniuiensis* by their shorter, wider conidia: *V. bambusicola* 8–45 $\times$ 4.5–9.5 μm (Dai & al. 2015); *V. breviconidiophora* 25.2–32.4 $\times$ 7.9–10.2 μm (Hyde & al. 2017); *V. camagueyensis* 45–65 $\times$ 4–6 μm (Castañeda-Ruiz & al. 2017); *V. khunkonensis* 17.5–35 $\times$ 6–10 μm (Dai & al. 2015); and *V. mahabaleshwarensis* 5–25 $\times$ 4–9 μm (Pratibha & Bhat 2008).

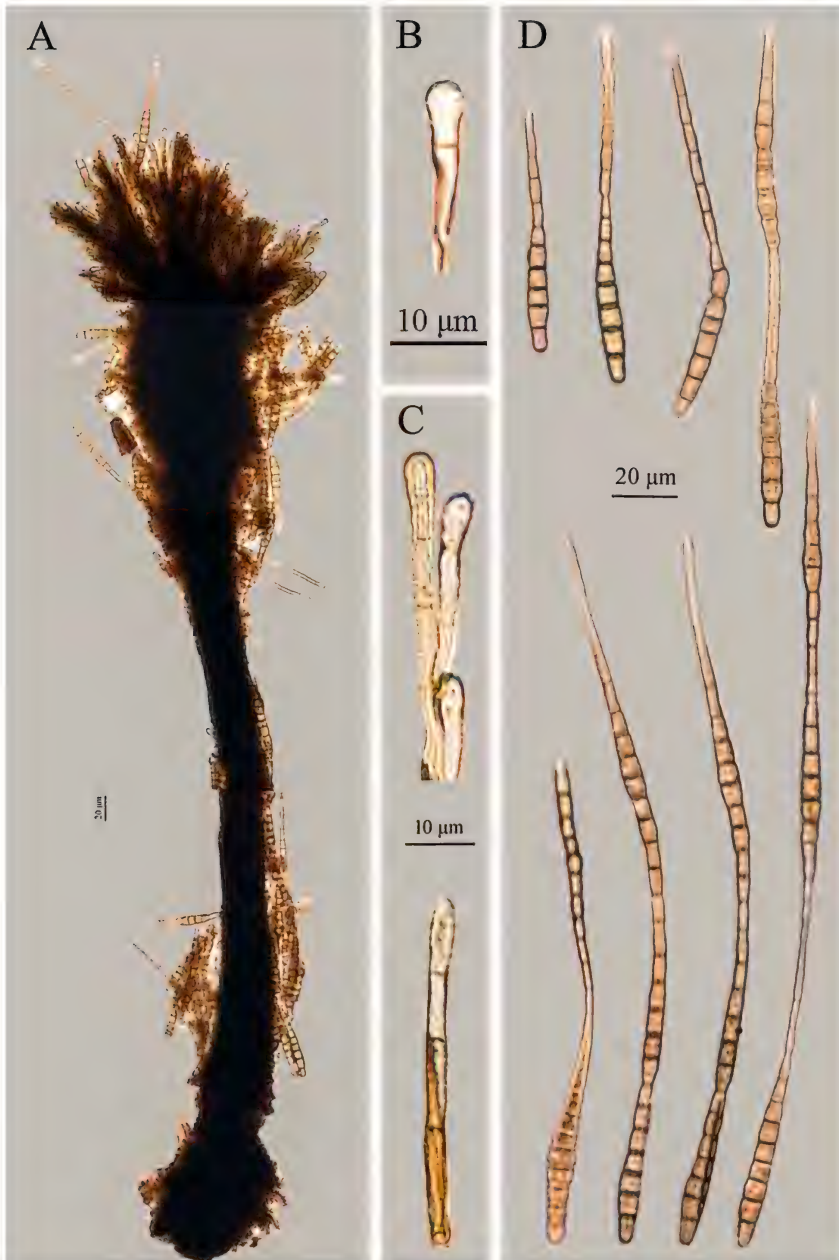


FIG. 1. *Vamsapriya jinniuensis* (holotype, HSAUP H10242). A. Synnema; B, C. Conidiophores and conidiogenous cells; D. Conidia.

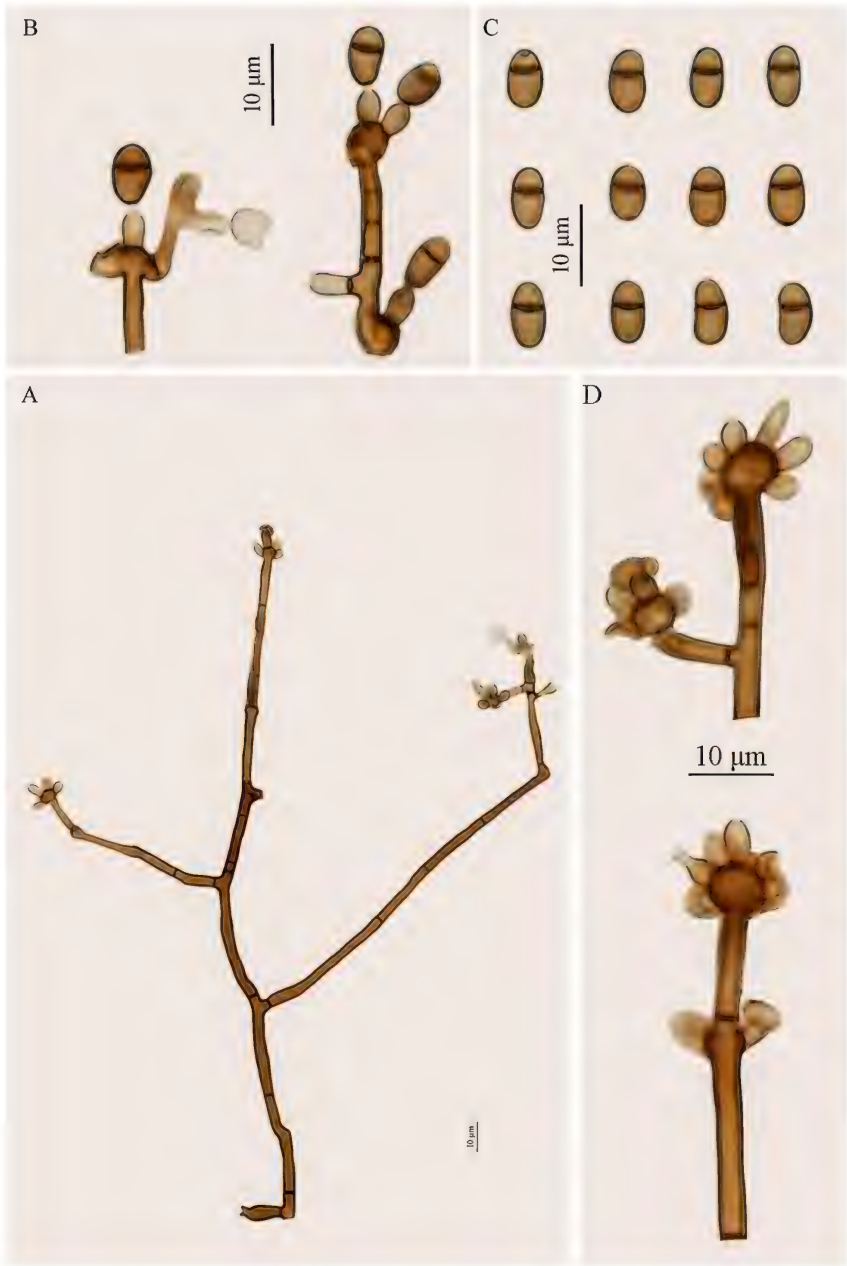


FIG. 2. *Garraudia elegans* (HSAUP H10237). A. Conidiophore, conidiogenous cells, and conidia; B. Conidiogenous cells and conidia; C. Conidia; D. Conidiogenous cells.

Garnaudia elegans Borowska, Acta Mycol. 13(1): 170, 1977.

FIG. 2

CONIDIOPHORES macronematous, mononematous, brown, becoming paler towards the apex, erect, flexuous, simple or branched, up to 237.5 µm long, 2.5–4 µm wide towards the base. CONIDIOGENOUS CELLS monoblastic, discrete, determinate, ampuliform or lageniform, truncated, subhyaline or pale yellowish brown, solitary or in groups, 4.5–5.6 µm long, 2–3 µm wide at the apex of each stipe or branch. CONIDIA solitary, dry, acrogenous, simple, ellipsoidal, oval or piriform, 1-septate, basal cell 2× longer than apical cell, evenly pigmented, smooth, 6.5–8.5 × 3–5 µm, sometimes with a basal hyaline marginal frill.

SPECIMEN EXAMINED: CHINA, JIANGXI PROVINCE: Dayueshan, on dead stems of an unidentified broadleaf tree, 23 Dec. 2016, H.H. Li (HSAUP H10237).

COMMENTS – *Garnaudia* was established by Borowska (1977) with *G. elegans* as type species. It is mainly characterized by macronematous, simple or branched conidiophores, and monoblastic, discrete, determinate, terminal and intercalary, verticillate, ampuliform or lageniform conidiogenous cells that produce solitary, acrogenous, simple, brown, continuous or septate conidia.

We report *Garnaudia elegans* for the first time from China. Our Chinese specimen is similar to the Polish protologue description, but its conidiophores are slightly shorter than those of the type material (≤300 µm; Borowska 1977).

Acknowledgments

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Rare or interesting lichen species new to China

QIANG REN* & XIAO-JIA ZHENG

College of Life Sciences, Shandong Normal University, Jinan 250014, China

*CORRESPONDENCE TO: rendaqiang@hotmail.com

ABSTRACT —During recent studies on the microlichens from Mount Taibai in Qinling Mountains of northwestern China, *Bryobilimbia hypnorum*, *Catillaria nigroclavata*, *Mycobilimbia tetramera*, *Placidiopsis pseudocinerea*, and *Toninia diffracta* were revealed as new records for China or mainland China. Illustrations and detailed taxonomic descriptions are provided for these five newly reported species.

KEY WORDS—Ascomycota, Catillariaceae, Porpidiaceae, Ramalinaceae, Verrucariaceae

Introduction

Mount Taibai, the highest mountain of the Qinling Range, is located in the southwest of the Shaanxi Province in China; its tallest point, the Baxian Tower, is 3767 m a.s.l. Due to its relatively high elevation, a typical vertical distribution of vegetation ranges can be observed. Thus, the Mount Taibai region houses plants from subtropical to warm temperate climates as well as some elements from middle temperate and alpine climates.

One hundred and sixty-three lichen species were reported from Mount Taibai by Guo (2005), and subsequently five new species of crustose lichens have been described from there (Ren 2013, 2014, 2017; Zhang & Ren 2016). Here we present five additional lichen records from Mount Taibai, of which four are new for China and one is new for the mainland. Illustrations accompany detailed morphological and chemical descriptions of the newly reported taxa.

Material & methods

The material was collected in the following vegetation types (see Liu & al. 2002): deciduous oak forests (with *Quercus variabilis*, *Q. aliena* var. *acutiserrata*, and *Q. liaotungensis*; 800–2300 m), birch forest (with *Betula albosinensis*; 2300–2730 m), coniferous forest zone (with *Abies fargesii*, *A. chinensis*, *A. sutchuensis*, and *Larix chinensis* [the latter forming the timber line]; 2730–3400 m), and *Kobresia*-dominated alpine meadows (3400–3700 m). The specimens examined are housed in the herbarium of Shandong Normal University, Jinan, China (SDNU). We examined the morphology using an Olympus SZ51 dissecting microscope and an Olympus CX21 compound light microscope and determined lichen secondary metabolites by spot test reagents and standardized thin-layer chromatography (TLC) with solvents A, B, & C (Elix 2014). Photographs were taken using an Olympus SZX16 stereomicroscope with a DP72 digital camera.

Taxonomy

Bryobilimbia hypnorum (Lib.) Fryday, Printzen & S. Ekman,

Lichenologist 46: 31 (2014).

FIG. 1

Thallus crustose, thin, grey. Photobiont green, globular, cells 10–17 µm diam.

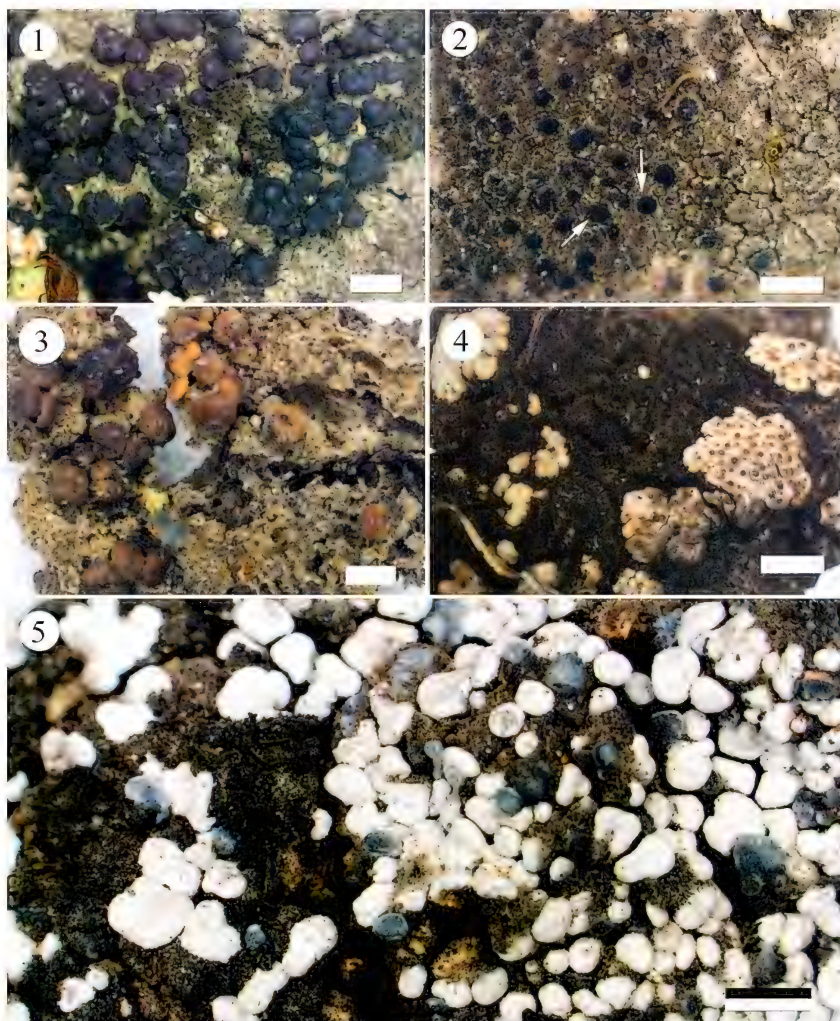
Apothecia single or sometimes clustered, dark brown to black, 0.5–1.1 mm diam.; disk flat to convex; exciple dark red-brown and persisting; epithecium pale brown; hymenium colorless, 75–87.5 µm tall; hypothecium dark red-brown, hymenium and hypothecium with blue-violet granules (K+ green); paraphyses 1–1.5 µm diam., simple, apices <2.5 µm diam., without dark brown cap; asci 8-spored, of the *Porpidia*-type; ascospores 1(–3)-septate, with warted epispore, colorless, ellipsoid, 12–16.5(–18) × 5.5–6 µm. Pycnidia not seen.

CHEMISTRY: Thallus K–, C–, KC–, PD–. No lichen substances detected by TLC.

SPECIMEN EXAMINED: CHINA. SHAANXI: Meixian County, Mount Taibai, on the roadside between Xiao Wengong Temple and Wengong Temple, alt. 3448 m, on moss, 12 Sep. 2017, Q. Ren 5105 (SDNU).

COMMENTS: *Bryobilimbia hypnorum* is a new record for China. Our Chinese specimen is closely similar to the specimens described by Fryday & al. (2014), which were characterized by a muscicolous thallus with single to clustered apothecia, blue-violet granules in the hymenium, 8-spored asci of the *Porpidia*-type, and colorless, often 1-septate ascospores with a very finely warted epispore.

Bryobilimbia sanguineoatra (Wulfen) Fryday & al. differs from *B. hypnorum* by its narrower ascospores (3–4.5 µm diam.; Fryday & al. 2014).



FIGS. 1–5. 1. *Bryobilimbia hypnorum*, thallus habit; 2. *Catillaria nigroclavata*, thallus habit (white arrows point to black apothecia); 3. *Mycobilimbia tetramera*, thallus habit; 4. *Placidiopsis pseudocinerea*, thallus habit; 5. *Toninia diffracta*, thallus habit. Scale bars: 1–3 = 1 mm; 4, 5 = 2 mm.

Catillaria nigroclavata (Nyl.) J. Steiner, Sitzungsber. Kaiserl. Akad. Wiss., Wien., Math.-Naturwiss. Cl., Abt. 1, 107: 157 (1898).

FIG. 2

Thallus crustose, thin, grey-brown, often inconspicuous, without a distinct margin. Verrucae granular, 0.04–0.1 mm diam. Soredia and isidia absent. Photobiont chlorococcoid; cells 10–12.5 µm diam.

Apothecia sessile, flat, black, 0.15–0.3 mm diam.; margin slightly raised, concolourous with disk; exciple brown at outer edge, colorless to pale brown at inner edge; epithecium brown, K–, N–; hymenium colorless, 37.5–42.5 µm tall; hypothecium pale brown; paraphyses 1–1.5 µm diam., simple or sparsely branched; apices swollen to 4 µm diam., with dark brown caps; asci 8-spored, of the *Catillaria*-type; ascospores 1-septate, colorless, ellipsoid, 7.5–11 × 2.5–3 µm. Pycnidia not seen.

CHEMISTRY: Chemistry unknown.

SPECIMENS EXAMINED: CHINA. SHAANXI: Meixian County, Mount Taibai, alt. 2250 m, on bark, 16 Jun. 2011, Y. Dong 20114251 (SDNU); alt. 2400 m, on bark, 16 Jun. 2011, Y.L. Cheng 20114329C-1 (SDNU).

COMMENTS: *Catillaria nigroclavata*, previously reported from Taiwan (Aptroot & Sparrius 2003), is here newly recorded from mainland China. Our specimens closely resemble those described by Fletcher & Coppins (2009), characterized by a corticolous thallus, paraphyses with swollen apices and a dark brown apical cap, an exciple with a brown outer edge, and 8-spored *Catillaria*-type asci.

Catillaria nigroclavata resembles *C. chalybeia* (Borrer) A. Massal. and *C. lenticularis* (Ach.) Th. Fr., but *C. chalybeia* has slightly larger apothecia (0.2–0.5 mm) and usually green pigments in the hymenium, and *C. lenticularis* differs by its saxicolous thallus and convex apothecia (Fletcher & Coppins 2009).

Mycobilimbia tetramera (De Not.) Vitik., Ahti, Kuusinen, Lommi & T. Ulvinen
ex Hafellner & Türk, Stapfia 76: 148 (2001). FIG. 3

Thallus crustose, thin or rather thick, grey-brown, coarsely granular or warted, without a distinct margin. Photobiont chlorococcoid.

Apothecia single, disc flat to slightly concave at first, becoming slightly convex when mature, grey-brown, orange-brown, or red-brown, 0.5–1.2 mm diam.; margin concolourous with disc or paler, at first distinct, level with disc, soon excluded and reflexed; exciple pale yellow, K+ intensifying, c. 62.5–90 µm diam.; epithecium slightly brown; hymenium colorless, 77.5–100 µm high; paraphyses simple or rarely branched, 1–1.5(–2) µm diam., apices slightly swollen; hypothecium colorless, or pale yellow, K+ intensifying; asci 8-spored, cylindrical, of the *Biatora*-type; ascospores colorless, 3-septate, fusiform, straight, 17.5–21 × 5–6 µm. Pycnidia not seen.

CHEMISTRY: Thallus K–, C–, KC–, PD–. No lichen substances detected by TLC.

SPECIMEN EXAMINED: CHINA. SHAANXI: Meixian County, Mount Taibai, Mingxing Temple, alt. 2900 m, on mosses over rock., 4 Aug. 2005, Y.J. Li & W. Fu L-179 (SDNU).

COMMENTS: *Mycobilimbia tetramera* is a new record for China. Our specimen closely resembles those described by Printzen & al. (2009), which were characterized by a coarsely granular or warted thallus on mosses over rock, grey- to red brown apothecia, 8-spored *Biatora*-type asci, and 3-septate ascospores.

Mycobilimbia tetramera is very similar to *M. carneoalbida* (Müll. Arg.) S. Ekman & Printzen and *M. epixanthoides* (Nyl.) Vitik. & al., but *M. carneoalbida* has whitish to pale orange-brown apothecia and shorter hymenium (65–75 µm), and *M. epixanthoides* differs by its sorediate thallus and infrequent apothecia (Printzen & al. 2009).

Placidiopsis pseudocinerea Breuss, Pl. Syst. Evol. 142: 248 (1983) FIG. 4

Thallus squamulose; squamules ≤4.2 mm across, closely appressed, scattered, pale brown, underside black. Photobiont green, cells globular, 7.5–10 µm diam.

Perithecia fully immersed, 0.2–0.32 mm diam., ostioles dark, ±elevated; true exciple colorless to black-brown; asci 8-spored, biseriate; ascospores 1-septate, colorless, ellipsoid, 15–17.5(–20) × (7–)7.5–8(–8.5) µm. Pycnidia not seen.

CHEMISTRY: Thallus K–, C–, KC–, PD–. No lichen substances detected by TLC.

SPECIMENS EXAMINED: CHINA. SHAANXI: Meixian County, Mount Taibai, Xiao Wengong Temple, alt. 3480 m, on soil, 11 Sep. 2017, Q. Ren 4719 (SDNU); Wengong Temple, alt. 3530 m, on soil, 12 Sep. 2017, Q. Ren 4909 (SDNU).

COMMENTS: *Placidiopsis pseudocinerea* represents a new record for China. Our specimens closely resemble those described by Breuss (2009) in producing a squamulose thallus on soil, scattered squamules c. 4 mm in diam., fully immersed perithecia, and 8-spored asci with 1-septate ascospores.

Placidiopsis pseudocinerea resembles *Catapyrenium cinereum* (Pers.) Körb., which differs by its smaller squamules (rarely >2 mm), and its simple and longer (17–23 µm) ascospores (Breuss 2009).

Toninia diffracta (A. Massal.) Zahlbr., Oesterr. Bot. Z. 51: 284 (1901). FIG. 5

Thallus squamulose, squamules scattered at first, becoming contiguous, weakly convex to hemispherical when mature; upper surface white-grey,

densely white-pruinose, pruina granular; the edges concolourous with upper side, the underside white to pale brown, 0.8–2 (–2.5) mm diam. Photobiont chlorococcoid; cells yellow-green, globular, 9–12 µm diam.

Apothecia 0.8–1.5(–2) mm diam., single, slightly concave to convex, black covered by white pruina; margin concolourous with disc; exciple brown, K+ violet, N+ violet; epithecium grey, K+ violet, N+ violet; hymenium colorless to slightly brown, 75–87.5 µm tall; paraphyses simple or rarely branched, 1.5–2.5 µm diam., apices swollen to 5 µm diam.; hypothecium brown, 50–62.5 µm tall; asci 8-spored, cylindrical, of the *Biatora*-type; ascospores colorless, 1-septate, fusiform, 15–20 × 3–4 µm. Pycnidia not seen.

CHEMISTRY: Thallus K–, C–, KC–, PD–. No lichen substances by TLC.

SPECIMEN EXAMINED: CHINA. SHAANXI: Meixian County, Mount Taibai, Wengong Temple, alt. 3660 m, on soil, 5 Aug. 2005, Y.J. Li & W. Fu L-390 (SDNU).

COMMENTS: *Toninia diffracta* represents a new record for China. Our specimen is closely similar to those described by Hitch & al. (2009), which were characterized by a squamulose thallus on soil, scattered to contiguous white-pruinose squamules, grey epithecium K+ violet, 8-spored *Biatora*-type asci, and 1-septate ascospores.

Toninia diffracta is very similar to *T. candida* (Weber) Th. Fr., which differs by its farinose pruina, a continuous rosette-shaped thallus, and always lacks regular fissures in the cortex (Hitch & al. 2009).

Acknowledgements

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***Planetella lironis* – a new record from Greenland**

TEODOR T. DENCHEV* & CVETOMIR M. DENCHEV

*Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences,
2 Gagarin St., 1113 Sofia, Bulgaria*

* CORRESPONDENCE TO: ttdenchev@gmail.com

ABSTRACT—*Planetella lironis*, a rare smut fungus known only from Canada, is reported for the first time from Greenland, on *Carex maritima*.

KEY WORDS—*Anthracoidea*, *Anthracoideaceae*, *Cyperaceae*, taxonomy

Introduction

The monotypic genus *Planetella* was introduced by Savile (1951) to accommodate a *Cintractia*-like (currently, *Anthracoidea*-like) smut fungus on two sedges in *Carex* subg. *Vignea*, forming sori around aborted nuts, as hard bodies covered by a thick peridium, and unusual for *Anthracoidea* spores with a thick-walled, dark equatorial band and two, thin-walled, light polar areas. *Planetella lironis* is known only from Canada on *Carex maritima* Gunnerus [= *C. incurva* Lightf.] from the type locality in Nunavut Province, and also on *C. sabulosa* Turcz. ex Kunth [= *C. leiophylla* Mack.] from Yukon Province (Savile 1951, Vánky 2013). As a closely related genus to *Anthracoidea*, *Planetella* is included in the *Anthracoideaceae* (Denchev 1997, Vánky 2013).

During an examination of sedge specimens in the Greenlandic herbarium, which is part of the herbarium of University of Copenhagen, Denmark (C), a sorus of *P. lironis* was found in a female flower of a specimen of *Carex maritima*. This specimen represents the first record of *P. lironis* from Greenland.

Materials & methods

A dried specimen from the herbarium of University of Copenhagen (C) was examined under light microscope (LM) and scanning electron microscope (SEM). For LM observations and measurements, spores were mounted in lactoglycerol solution (w : la : gl = 1 : 1 : 2) on glass slides, gently heated to boiling point to rehydrate the spores, and then cooled. The spore measurements are given as min–max (mean $\pm$ standard deviation). For SEM, spores were attached to specimen holders by double-sided adhesive tape and coated with platinum in an ion sputter. The surface structure of spores was observed and photographed at 10 kV accelerating voltage using a JEOL JSM 6610-LV scanning electron microscope (Natural History Museum Vienna, Austria). The height of warts was measured in SEM. The type of spore ornamentation, as seen by SEM, is in accordance with Denchev & al. (2013). The description below is based entirely on the specimen examined. The list of shapes of spores is arranged in descending order of frequency.

Taxonomy

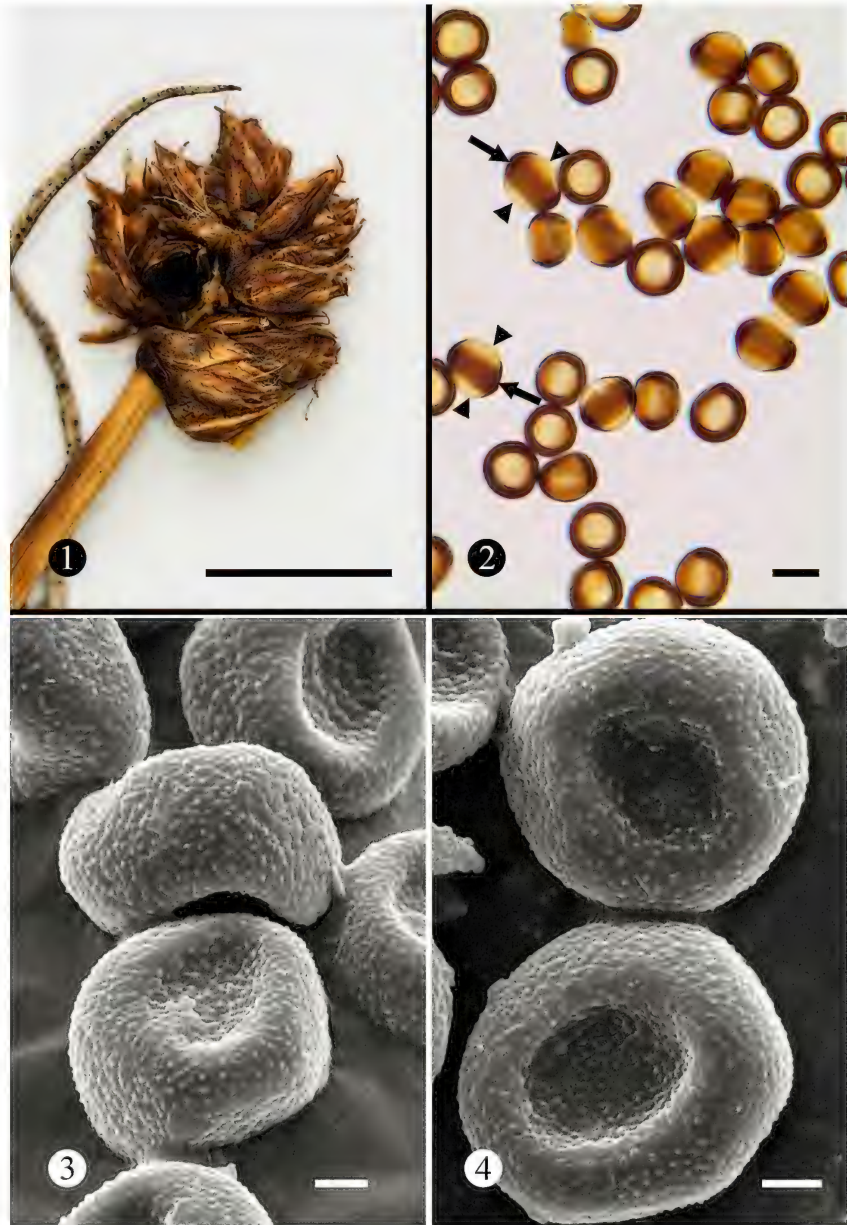
Planetella lironis Savile, Canad. J. Bot. 29: 327, 1951.

FIGS 1–4

SORI in some female flowers, around aborted nuts, as subglobose, hard bodies, ca. 2 mm long, covered by a thick, yellow-brown peridium that later flakes away exposing a black, agglutinated (semi-agglutinated on the surface) spore mass. SPORES slightly flattened, with a thick-walled, medium reddish brown equatorial band and two, thin-walled, light yellow-brown polar areas; in plane view suborbicular, orbicular, broadly elliptical or slightly irregularly rounded, in plane view (10.5–)11–13.5(–14.5) $\times$ (9.5–)10–12.5(–13) μm ($12.1 \pm 0.6 \times 11.2 \pm 0.6 \mu\text{m}$) ($n = 100$); equatorial band 6.0–8.2 μm wide; in plane view polar areas suborbicular, orbicular, elliptical, broadly elliptical or slightly irregularly rounded, 5.5–7.5(–8.5) μm long; wall unevenly thickened, (1.5–)1.7–2.5(–2.7) μm thick at the equatorial band, 0.5–1.0(–1.2) μm thick at the polar areas, minutely verruculose, spore profile not affected. In SEM spore walls minutely verruculose; warts densely spaced, less than 0.2 μm in height, usually isolated. SPORE GERMINATION unknown.

SPECIMEN EXAMINED—On *Carex maritima*: GREENLAND, AVANNAATA, Nuussuaq Peninsula (as ‘Nügssuaq Pen.’), Kûtsiaq, 70°40’N 52°27’W, 19 August 1947, leg. T. Sørensen, Danish Botanical Expedition to West Greenland 1947, no. 9196 (C).

COMMENTS—The new location of *Planetella lironis* extends the eastern and northern limits of this species and its geographic range from Yukon through Nunavut to West Greenland (FIG. 5). Although the range of *P. lironis* seems large, this fungus is known only from single localities. On *Carex maritima*,



FIGS 1–4. *Planetella lironis* on *Carex maritima* (herb. C). 1. Habit. 2. Spores in LM (arrows indicate spore equatorial band, arrowheads show spore polar areas). 3, 4. Spores in SEM. Scale bars: 1 = 0.5 cm; 2 = 10 µm; 3, 4 = 2 µm.



FIG. 5. Geographic distribution of *Planetella lironis* (red circles – on *Carex maritima*; blue circle – on *C. sabulosa*) (generated with SimpleMappr, Shorthouse 2010). Scale bar = 500 km.

P. lironis has been previously recorded only from the type locality (Nunavut, Chesterfield Inlet, 63°21'N 90°42'W; Savile 1951). *Carex maritima* is a widespread species, distributed in Alaska, Canada, Greenland, and North Eurasia (having a circumpolar range in the Northern Hemisphere), and in alpine regions of Europe and Central Asia, as well as in South America (from Ecuador to Argentina) (Egorova 1999, Reznicek 2002, Villaverde & al. 2015, Govaerts 2018). It is noteworthy that *P. lironis* is not reported from North Europe and the alpine regions of Central Europe, which are among the best studied regions in the world for smut fungi, i.e. its absence there is not due to inadequate studies. *Planetella lironis* is a remarkable example of a smut fungus with restricted distribution although its principal host is a widespread plant species.

This statement is also true with regard to occurrence on *Carex sabulosa* which has very restricted distribution in North America, known from only 14 localities in Yukon and one in Alaska (Murray 2002, Baikal Sedge Recovery Team 2012). *Carex sabulosa* is also known from East Siberia,

Kazakhstan, and North Mongolia (Egorova 1999). *Planetella lironis* is found at most localities of *C. sabulosa* in Yukon (Baikal Sedge Recovery Team 2012) (Fig. 5) but has never been found in Asia.

In Greenland, the occurrence of a smut fungus on *Carex maritima* was reported by Allescher & Hennings (1897; as *Cintractia caricis* on *Carex incurva*, Uummannaq Island, Uummannaq, as 'Umanak', 28 June 1892). There is no information that this specimen is kept at any mycological collection (see Savile 1951). This record was provided with a very short description, making the identification of the fungal species impossible, although one can assume that it was a specimen of *Anthracoidea* based on the size of spores, given by Allescher & Hennings (1897) as 18–22 µm in diam. Nannfeldt (1979), without seeing this specimen, assigned it to *Anthracoidea* sp. no. 5 on *Carex maritima*. Thus, the specimen of *Planetella lironis* reported here is a first record of this species from Greenland.

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New records of smut fungi from Korea. 3

TEODOR T. DENCHEV^{1*}, HYEON-DONG SHIN², CVETOMIR M. DENCHEV¹

¹ Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences,
2 Gagarin St., 1113 Sofia, Bulgaria

² Division of Environmental Science and Ecological Engineering, Korea University,
Seoul 02841, South Korea

* CORRESPONDENCE TO: ttdenchev@gmail.com

ABSTRACT—Four smut fungi, *Microbotryum nepalense*, *Sphacelotheca polygoni-senticosi*, *Tilletia barclayana*, and *T. setariae-viridis*, are reported for the first time from South Korea. Additionally, *Persicaria longiseta* and *P. posumbu* are newly recorded as South Korean hosts of *Sphacelotheca hydropiperis*, and *Echinochloa crus-galli* is a new South Korean host for *Ustilago trichophora*.

KEY WORDS—*Microbotryaceae*, *Poaceae*, *Polygonaceae*, taxonomy, *Tilletiaceae*

Introduction

As part of an ongoing study of smut fungi in South Korea (Denchev & Kakishima 2007; Denchev & al. 2007a, b, 2013; Park & al. 2010; Denchev & Denchev 2014), a taxonomic revision of specimens revealed four species new for this country: *Microbotryum nepalense*, *Sphacelotheca polygoni-senticosi*, *Tilletia barclayana*, and *T. setariae-viridis*, which are reported herein.

Materials & methods

Dried specimens from the Mycological Herbarium of Korea University, Seoul (KUS-F), College of Natural Sciences, Seoul National University, Seoul (SNU), and C.M. Denchev's personal collection (CMD) were examined under light microscope (LM) and scanning electron microscope (SEM). For LM observations and measurements, spores were mounted in lactoglycerol solution (w : la : gl = 1 : 1 : 2) on glass slides, gently heated to boiling point to rehydrate the spores, and then cooled.

The spore measurements are given as min–max (mean $\pm$ standard deviation). For SEM, spores were attached to specimen holders by double-sided adhesive tape and coated with platinum in a Hitachi E-1010 ion sputter. The surface structure of spores was observed and photographed at 15 kV accelerating voltage using a Hitachi S-3500N scanning electron microscope. The descriptions below are based entirely on the specimens examined. The list of shapes of spores is arranged in descending order of frequency.

Taxonomy

Microbotryum nepalense (Liro) Vánky, Mycotaxon 67: 47, 1998. FIGS 1, 2

SORI in the stem, fusiform, ≤ 5 cm long, usually in the upper part of the stem, most commonly closely below the intact inflorescence; initially covered by the epidermis that ruptures longitudinally exposing the semi-agglutinated, dark purple spore mass surrounding numerous vascular bundles. SPORES subglobose, broadly ellipsoidal, ellipsoidal, slightly irregular or ovoid, $(9.5\text{--})10\text{--}14.5(\text{--}15.5) \times (9\text{--})9.5\text{--}12.5(\text{--}13) \mu\text{m}$ ($12.2 \pm 1.0 \times 10.8 \pm 0.8 \mu\text{m}$) ($n = 100$), medium reddish brown; spore wall evenly thickened, $0.7\text{--}1.0 \mu\text{m}$ thick, completely or often incompletely reticulate; meshes $(8\text{--})9\text{--}14(\text{--}16)$ per spore diameter, polyhedral or irregular, $0.4\text{--}1.5(\text{--}3.2) \mu\text{m}$ long; muri low, spore profile slightly affected. Immature spores abundant, intermixed with mature ones, hyaline to light purplish brown, usually smaller than the mature spores. In SEM spore wall reticulate, muri low, up to $0.2 \mu\text{m}$ high; the interspaces usually with 1–6 warts.

SPECIMEN EXAMINED—On *Persicaria nepalensis* (Meisn.) H. Gross: SOUTH KOREA, GANGWON PROVINCE, Gangneung, 28 October 1992, leg. H.D. Shin (KUS-F 12312).

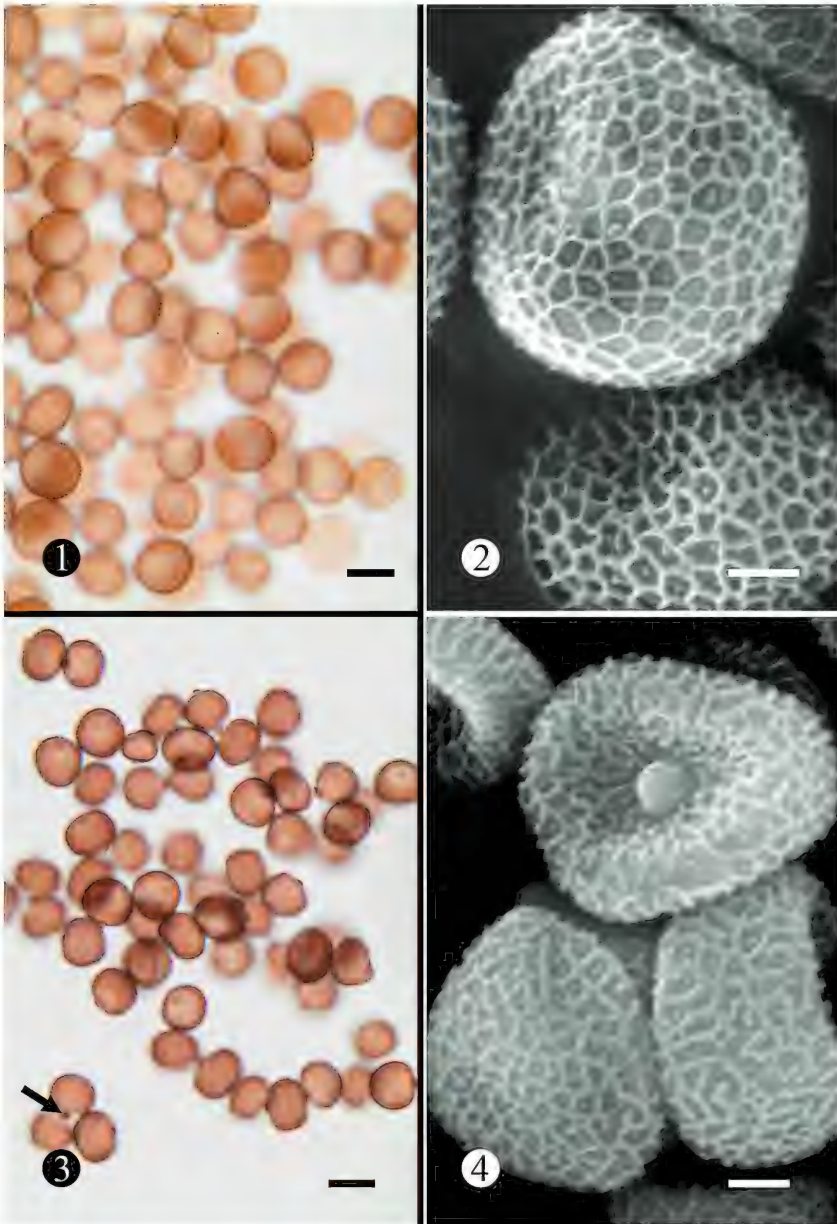
COMMENTS—The morphological features of the examined specimen are similar to those reported by Kakishima & al. (2000) and Vánky (2011).

Microbotryum nepalense is known from Asia (Tadzhikistan, Pakistan, India, Nepal, China, Russian Far East, and Japan) and East Africa (Ethiopia) (Ahmad 1956; Kakishima 1982; Karatygin & Azbukina 1989; Kakishima & Ono 1993; Azbukina & al. 1995; Guo 2000; Kakishima & al. 2000; Vánky 2005, 2007; Vánky & al. 2011; Denchev & Denchev 2017). *Persicaria nepalensis* is the only known host plant of this smut fungus.

Sphacelotheca polygoni-senticosi S. Ito, Trans. Sapporo Nat. Hist. Soc. 14: 90, 1935.

FIGS 3, 4

SORI in some ovaries of an inflorescence, swollen, 3.5–6.5 mm long, covered by a thick, brittle, yellow-brown peridium that later ruptures irregularly from the apex, exposing powdery, very dark reddish brown mass of spores,



FIGS 1–4. *Microbotryum nepalense* on *Persicaria nepalensis* (KUS-F 12312). 1. Spores in LM. 2. Spores in SEM. *Sphacelotheca polygoni-senticosi* on *Persicaria senticosa* (KUS-F 10409). 3. Spores in LM (arrow shows spores connected by a disjunct). 4. Spores in SEM. Scale bars: 1, 3 = 10 μ m; 2, 4 = 2 μ m.

surrounding a stout columella. SPORES often more or less flattened, in plane view suborbicular, slightly irregular, orbicular or broadly elliptical, in plane view $(9-9.5-12(-13) \times (8.5-9-11.5(-12) \mu\text{m})$ ($10.6 \pm 0.6 \times 10.2 \pm 0.5 \mu\text{m}$) ($n = 100$), middle purplish brown, initially in chains, connected by disjunctors, later single, with 2(-3), persistent appendages representing remnants of disjunctors; wall evenly thickened, $0.6-0.9 \mu\text{m}$ thick. In SEM the majority of warts variously interconnected, forming a labyrinthiform or incompletely reticulate surface pattern.

SPECIMENS EXAMINED—On *Persicaria senticosa* (Meisn.) H. Gross ex Nakai: **SOUTH KOREA, GANGWON PROVINCE, Yangku**, 7 June 1998, leg. H.D. Shin (KUS-F 14613); **Chuncheon**, 27 September 1997, leg. H.D. Shin (KUS-F 14271); 19 August 1998, leg. H.D. Shin (KUS-F 14847); 21 August 2001, leg. H.D. Shin (KUS-F 18262); 17 September 2001, leg. H.D. Shin (KUS-F 18421); 11 October 2002, leg. H.D. Shin (KUS-F 19213); **Gangneung**, 15 September 1990, leg. H.D. Shin (KUS-F 10409); 7 August 1993, leg. H.D. Shin (KUS-F 12506); **Hoengseong**, 11 October 2004, leg. H.D. Shin (KUS-F 20836); **Wonju**, 17 September 1999, leg. H.D. Shin (KUS-F 16749). **GYEONGGI PROVINCE, Mt. Hwaya**, Cheolgol Valley, alt. 160 m, 15 September 1996, leg. S.T. Kim (SNU 86594); **Gwangju**, 10 June 1972, sine coll. (SNU s.n.). **JEOLLANAM PROVINCE, Kurye**, Mt. Chiri at Hwaom Temple, 2 October 1999, leg. C.W. Park & J.H. Park (SNU 92095). **JEJU PROVINCE, Jeju**, 31 October 2002, leg. H.D. Shin (KUS-F 19325).

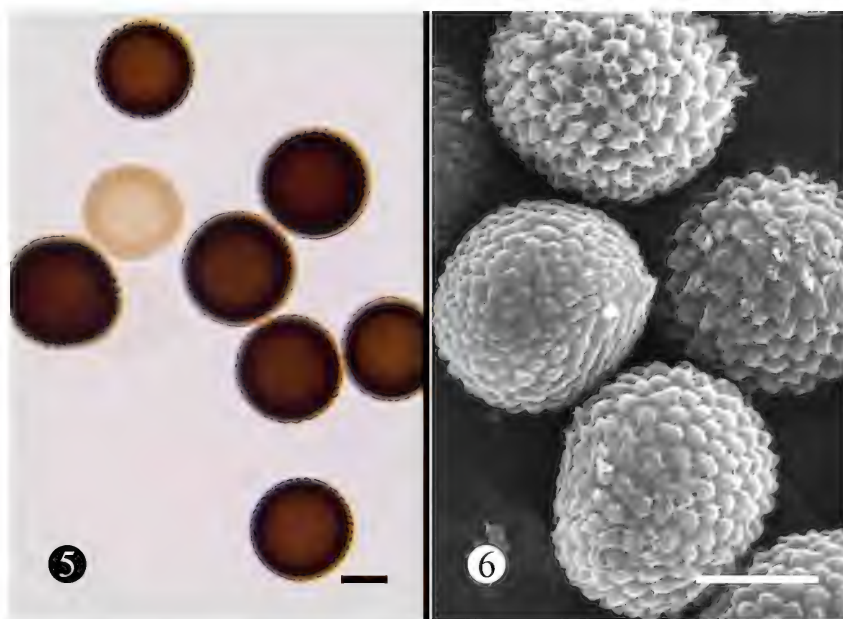
COMMENTS—Vánky & Oberwinkler (1994) and Vánky (2011) treated *S. polygoni-senticosi* as a synonym of *S. hydropiperis*, but we recognize it as a distinct species. The taxonomic status of *S. polygoni-senticosi* will be discussed in a separate article. The morphological description of *S. polygoni-senticosi*, given by Ito (1935, 1936), lacks information currently considered as essential for interpretation of *Sphacelotheca* species, but the morphological features of the examined Korean specimen match very well those of the holotype specimen, studied by us.

As a distinct species, *S. polygoni-senticosi* has been previously reported from Japan (Yoshinaga 1901, as *Ustilago polygoni-senticosi*; Ito 1935, 1936), Taiwan (Sawada 1944), and China (Guo 1992, Kakishima & al. 2000).

Tilletia barclayana (Bref.) Sacc. & P. Syd., Syll. Fung. 14: 422, 1899.

FIGS 5, 6

SORI in some swollen ovaries of an infected plant, ovoid, 4–6 mm long, with a short, acute tip, bearing a rudimentary style and stigmas, covered by a thick, initially greenish, later yellow-brown pericarp that ruptures from its apex exposing a powdery, blackish brown mass of spores and sterile cells. **STERILE CELLS** variable in shape, size, and wall thickness, subglobose, globose, broadly ellipsoidal or irregular, $11-27 \mu\text{m}$ long, hyaline or subhyaline; cell wall $1.6-3.5 \mu\text{m}$ thick, sometimes indistinctly laminate, occasionally with a narrow appendage.



FIGS 5, 6. *Tilletia barclayana* on *Pennisetum alopecuroides* (KUS-F 10405). 5. Spores in LM. 6. Spores in SEM. Scale bars = 10 μ m.

SPORES globose, subglobose or broadly ellipsoidal, (20.5–)21.5–29(–30.5) $\times$ (19–)20–26.5(–28.5) μ m ($24.2 \pm 1.8 \times 23.4 \pm 1.7 \mu$ m) ($n = 100$), middle to very dark reddish brown; spore wall (2.6–)2.8–3.7(–4.0) μ m thick (including the 0.8–1.8(–2.2) μ m high ornamentation), papillate; papillae (30–)32–42(–44) on equatorial circumference, in surface view solitary or appearing aggregated (seen as irregular, darker areas). In SEM papillae connected with low, fine ribs.

SPECIMENS EXAMINED—On *Pennisetum alopecuroides* (L.) Spreng.: **SOUTH KOREA**, GANGWON PROVINCE, Goseong, 27 August 2001, leg. H.D. Shin (KUS-F 18300); Gangneung, 15 September 1990, leg. H.D. Shin (KUS-F 10405). **INCHEON METROPOLITAN CITY**, Ganghwa Island, 28 September 1999, leg. H.D. Shin (KUS-F 16834).

COMMENTS—The morphological features of the examined specimens are similar to those reported by Vánky (2011).

An inner layer of the spore wall, that is 0.7–1.1 μ m thick, can be observed in the lightly colored, immature spores. *Tilletia barclayana* is known on *Pennisetum* spp. from Asia and North America (Vánky 2011).

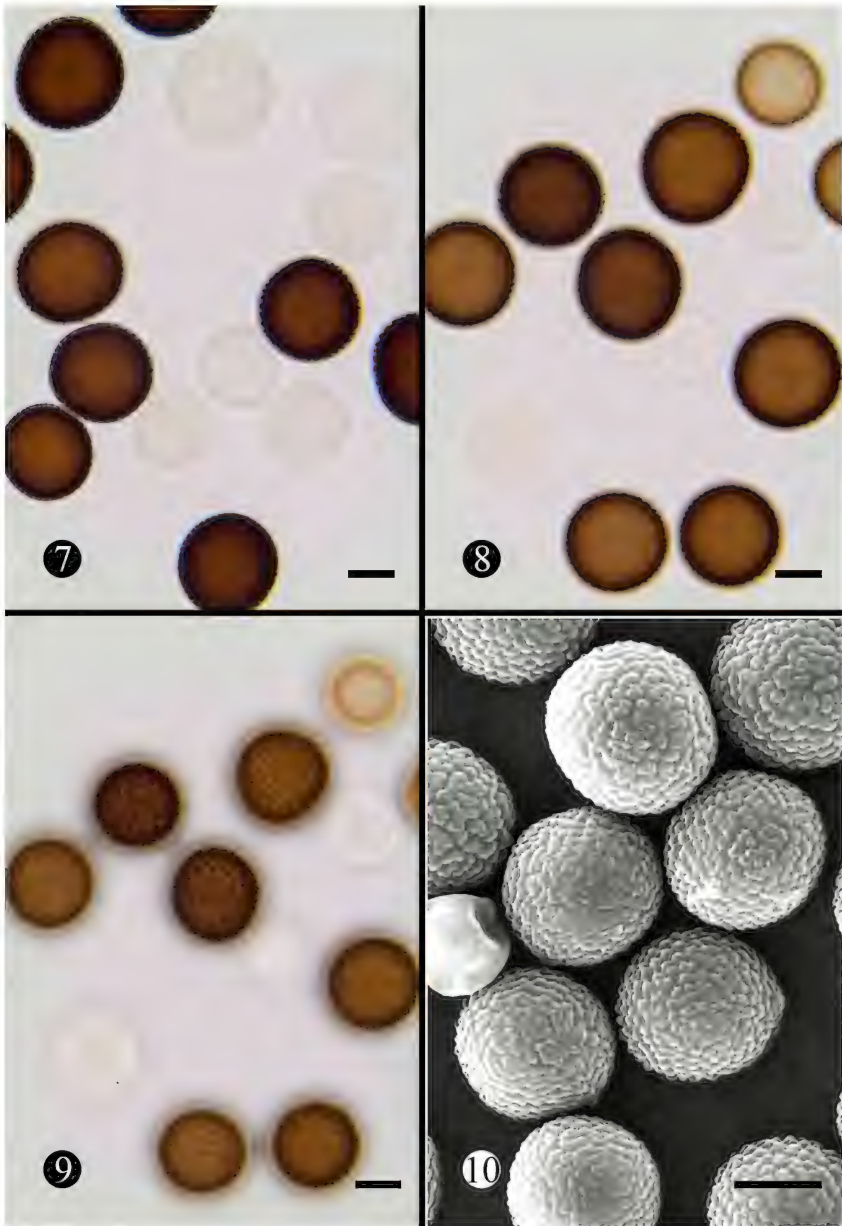
Tilletia setariae-viridis Vánky, Mycotaxon 78: 316, 2001.

FIGS 8–10

SORI in some swollen ovaries of an infected plant, exceeding the floral bracts, ovoid or ellipsoid, 2.5–4.5 mm long, sometimes bearing rudimentary stigmas, covered by a thick, initially greenish, later yellow-brown or purplish brown pericarp that ruptures from its apex exposing a powdery, blackish mass of spores and sterile cells. STERILE CELLS subglobose, slightly irregularly rounded or broadly ellipsoidal, $(9.5\text{--})11\text{--}23(\text{--}24) \times (9.5\text{--})10.5\text{--}22(\text{--}23) \mu\text{m}$, hyaline; cell wall two-layered or indistinctly laminate, $(1.2\text{--})1.4\text{--}2.6(\text{--}3.0) \mu\text{m}$ thick, sometimes with a narrow appendage, in SEM punctate. SPORES globose, subglobose or broadly ellipsoidal, $(21.5\text{--})22\text{--}27(\text{--}28) \times (20\text{--})21\text{--}25(\text{--}26) \mu\text{m}$ ($24.4 \pm 1.3 \times 23.2 \pm 1.2 \mu\text{m}$) ($n = 100$), medium or dark reddish brown; spore wall $(2.9\text{--})3.1\text{--}3.6(\text{--}3.8) \mu\text{m}$ thick (including the $0.7\text{--}1.2(\text{--}1.4) \mu\text{m}$ high ornamentation and the hardly visible, $0.7\text{--}1.0 \mu\text{m}$ thick inner layer), papillate, occasionally with a narrow, hyaline appendage; papillae in surface view often appearing aggregated (seen as irregular, darker areas; FIG. 9). In SEM papillae connected with low, fine ribs.

SPECIMENS EXAMINED—On *Setaria viridis* (L.) P. Beauv.: SOUTH KOREA, GANGWON PROVINCE, near Seom River, near Hoengseong Recreational Forest near Hoengseong town, $37^{\circ}32'31''\text{N}$ $128^{\circ}06'25''\text{E}$, 8 September 2006, C.M. Denchev 06-23 (CMD); Hoengseong Recreational Forest near Hoengseong town, $37^{\circ}32'13''\text{N}$ $128^{\circ}06'57''\text{E}$, 8 September 2006, C.M. Denchev 06-25 (CMD); Mt. Chiak near Wonju city, $37^{\circ}23'58''\text{N}$ $128^{\circ}02'59''\text{E}$, 15 September 2006, C.M. Denchev 06-16 (CMD). — CHINA, BEIJING, $39^{\circ}46' \text{N}$, $116^{\circ}20' \text{E}$, alt. c. 50 m, 22 September 1979, Z.Y. Zhao & al. (isotype, Vánky, Ustilag. Exsic. no. 292, as *T. setariae*).

COMMENTS—The above description of *Tilletia setariae-viridis* is based on the examined South Korean specimens. An isotype of *T. setariae-viridis* was also studied, as a comparative specimen (FIG. 7). The spore sizes of the Korean specimens are similar to those of the isotype, which measured $(20\text{--})21.5\text{--}27(\text{--}28) \times (20\text{--})21\text{--}25(\text{--}26) \mu\text{m}$ ($23.3 \pm 1.4 \times 22.2 \pm 1.1 \mu\text{m}$) ($n = 100$). Although the spore colour of *T. setariae-viridis* is given in its protologue (Vánky 2001) as yellowish brown, it is in fact medium or dark reddish brown. In the protologue, the spore wall thickness is measured without considering the thickness of the inner layer. The spore wall thickness of the Korean specimens is similar to that of the examined isotype. The protologue cites the height of the spore wall ornaments as $1.5\text{--}2.5 \mu\text{m}$, but in the isotype they measured $0.6\text{--}1.2 \mu\text{m}$, which matches that of the Korean specimens. The sterile cell sizes of the isotype are similar to those of the Korean specimens. The single sterile cells longer than $30 \mu\text{m}$ observed in both isotype and Korean specimens were considered aberrant and not included in the length range.



FIGS 7–10. *Tilletia setariae-viridis* on *Setaria viridis*. 7. Spores in LM (isotype, Vánky, Ustilag. Exsic., no. 292). 8, 9. Spores in LM (8 = median view; 9 = surface view; CMD 06-23). 10. Spores in SEM (CMD 06-23). Scale bars = 10 μ m.

Tilletia setariae-viridis has been previously known only from China (Vánky 2001, 2011; Guo 2011).

***Sphacelotheca hydropiperis* (Schumach.) de Bary,**

Vergl. Morph. Biol. Pilze: 187, 1884.

SPECIMENS EXAMINED—On *Persicaria longiseta* (Bruijn) Kitag.: **SOUTH KOREA, GANGWON PROVINCE, Hoengseong**, 11 October 2004, leg. H.D. Shin (KUS-F 20835, host as *P. blumei*).

On *Persicaria posumbu* (Buch.-Ham. ex D. Don) H. Gross: **SOUTH KOREA, GANGWON PROVINCE, Hongcheon**, 1 October 2003, leg. H.D. Shin (KUS-F 19778, host as *P. posumbu* var. *laxiflora*); **Jeongseon**, 16 October 2000, leg. H.D. Shin (KUS-F 17847, host plant as *P. posumbu* var. *laxiflora*).

COMMENTS—*Persicaria longiseta* and *P. posumbu* are new South Korean hosts of *Sphacelotheca hydropiperis*.

***Ustilago trichophora* (Link) Körn., Hedwigia 16: 36, 1877.**

SPECIMEN EXAMINED—On *Echinochloa crus-galli* (L.) P. Beauv.: **SOUTH KOREA, GANGWON PROVINCE, Yangku**, 28 September 1996, leg. H.D. Shin (KUS-F 13693).

COMMENTS—*Echinochloa crus-galli* is a new South Korean host of *Ustilago trichophora*; the smut has been previously reported from South Korea only on *E. frumentacea* Link (as *E. crus-galli* var. *frumentacea*; Cho & Shin 2004).

Acknowledgements

This study was supported by the Korea Research Foundation and the Korean Federation of Science and Technology Societies Grant funded by Korea Government (MOEHRD, Basic Research Promotion Fund). The authors gratefully acknowledge Dr Eric H.C. McKenzie (Manaaki Whenua Landcare Research, Auckland, New Zealand) and Dr Makoto Kakishima (University of Tsukuba, Tsukuba, Japan, and Jilin Agricultural University, Changchun, China) for critically reading the manuscript and serving as pre-submission reviewers, and Director and Curator of SNU (Herbarium of College of Natural Sciences, Seoul National University) for granting access to the collections.

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Myxomycetes from China 17: *Fuligo laevis* and *Physarum simplex*, newly recorded in China

FENGYUN ZHAO, YU LI, XINXIN GAO, SHUYAN LIU*

Engineering Research Center of Chinese Ministry of Education for
Edible and Medicinal Fungi, Jilin Agricultural University,
2888 Xincheng Street, Changchun City, China

* CORRESPONDENCE TO: liussyan@163.com

ABSTRACT—Two species new for China, *Fuligo laevis* and *Physarum simplex*, are herein described and illustrated with microscopic and scanning electronic micrographs. The specimens were collected in Heilongjiang Province and deposited in the Herbarium of Mycological Institute of Jilin Agricultural University, Changchun, China (HMJAU).

KEY WORDS—*Physaraceae*, taxonomy, plasmodial slime moulds

Introduction

Two new Chinese records of species of *Physaraceae*, *Fuligo laevis* and *Physarum simplex*, were found during the examination of our previous myxomycete collections. *Fuligo* is a common genus comprising ten species (Lado 2018), of which seven have been recorded in China (Li 2007, Li & al. 2017). *Physarum*, the largest genus in *Physaraceae*, comprises 142 species (Lado 2018), of which 75 have been recorded in China (Li 2007, Schnittler & al. 2013).

Materials & methods

Specimens of the two newly recorded species were collected in Heilongjiang Province and deposited in the Herbarium of the Mycological Institute of Jilin Agricultural University, Changchun, China (HMJAU).

Test materials on glass slides were wetted with absolute alcohol; one drop of 3% KOH solution was added to cause the spores and other structures to assume plumpness (correcting the contraction effect of alcohol) before the alcohol was completely

evaporated; finally, a drop of 8% glycerin was added and a cover glass placed over the rehydrated material prior to microscopical examination (Martin & Alexopoulos 1969). The tissues were observed and photographed using a Zeiss Imager A2 light microscope and a Hitachi SU8010 scanning electron microscope (SEM). For SEM, the dried samples were sputter-coated with gold-palladium to form a 5 nm cover and examined at 5–15 kV.

Taxonomy

Fuligo laevis Pers., Syn. Meth. Fung. 1: 160 (1801)

PL. 1

AETHALIUM irregular, pulvinate, about 2.5 cm in cross section and 1 cm thick, pale yellow. CORTEX persistent, almost smooth. HYPOTHALLUS cartilaginous, perforated, pale yellow. PSEUDOCAPILLITIUM branched, impregnated with pale lime. CAPILLITIUM slender, connected to the pseudocapillitium, with pale brown or pale yellow spindle-shaped lime nodes. SPORES free, mass dark, globose, 7.8–8.5 μm in diam., with minute and dense warts.

SPECIMEN EXAMINED: CHINA, HEILONGJIANG PROVINCE, Yichun, Liangshui National Nature Reserve, 46°43'48"N 129°54'0"E, 104 m, on the bark surface of dead log, 26 August 2014, Fengyun Zhao, SM2014082616 (HMJAU1017).

COMMENTS: *Fuligo laevis* has been recorded from Europe and USA (Poulain & al. 2011). It is similar to *F. leviderma* H. Neubert & al., but its aethalium is pale yellow in color, and smaller in size (Poulain & al. 2011). The first report of *Fuligo leviderma* from China was from Jilin Province (Li & al. 2017), and we have found it also growing on the bark of a stump in Heilongjiang

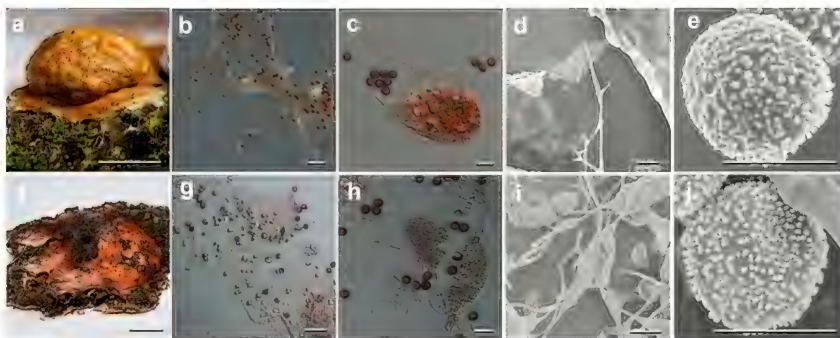


PLATE 1. Morphological comparison of *Fuligo laevis* (HMJAU1017) and *F. leviderma* (HMJAU1018). *Fuligo laevis*: a. Aethalium; b. Pseudocapillitium under microscope; c. Capillitium with lime-knot under microscope; d. Capillitium with lime-knot under SEM; e. Spores with warts under SEM. *Fuligo leviderma*: f. Aethalium; g. Pseudocapillitium under microscope; h. Capillitium with lime-knots under microscope; i. Capillitium with lime-knots under SEM; j. Spores with warts under SEM. Scale bars: a, f = 1 cm; b, g = 50 μm ; c, h = 20 μm ; d, i = 10 μm ; e, j = 4 μm .

Province [50°00'36"N 127°16'48"E, 130 m, 17 September 2014, Fengyun Zhao, SM2014091717 (HMJAU1018)]. *Fuligo laevis* and *F. leviderma* are compared morphologically in PLATE 1.

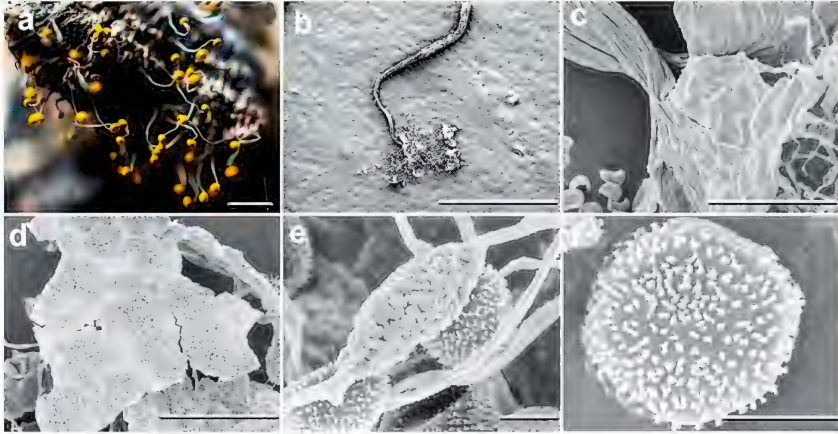


PLATE 2. *Physarum simplex* (HMJAU1019): a. Fruiting bodies growing on decaying bark; b. Stalk with capillitia; c. Stalk connected with peridium; d. Peridium covered with flakes of lime; e. Capillitium with lime node; f. Spores with warts. Scale bars: a, b = 1 mm; c, d = 40 μ m; e, f = 4 μ m.

Physarum simplex M. Peck, Amer. J. Bot. 19: 136 (1932)

PL. 2

SPOROCARPS stipitate, globose, minute, 0.15–0.25 mm in diam., yellowish. STALK very long, 1.5–2 mm, slender, narrowed upward, grooved, often crooked and drooping above, light grey, little or incomplete calcareous. HYPOTHALLUS none. PERIDIUM thin, often strewn with abundant flakes of lime. COLUMELLA none. CAPILLITIUM very delicate threads springing from the peridium, with a few yellowish lime nodes. LIME NODES scanty, fusiform. SPORES violaceous brown, warted, 8–10 μ m in diam.

SPECIMEN EXAMINED: CHINA, HEILONGJIANG PROVINCE, Jiamusi, Daliangzihe Park, 47°01'48"N 129°40'48"E, 109 m, on the bark of dead log, 25 August 2014, Shuyan Liu & Fengyun Zhao, SM2014082521 (HMJAU1019).

COMMENTS: *Physarum simplex* was originally described from the USA (Lado 2018). It is morphologically similar to *P. flavicomum* Berk. and *P. viride* (Bull.) Pers., but the peridium of *P. flavicomum* is nearly limeless, and its stalk is reddish brown, capillitium persistent, sometimes entirely reticulate, lime nodes scanty, irregular (Poulain & al. 2011). The sporocysts of *P. viride* are subglobose or flattened at base, and its stalk is pale yellowish or reddish, and partially near black; the lime nodes are usually elongated and fusiform (Poulain & al. 2011).

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***Cystotheca kusanoi* comb. nov.: a redescription with new morphological observations**

SUNG-EUN CHO¹, SUSUMU TAKAMATSU²,
SANG HYUN LEE³, HYEON-DONG SHIN^{1*}

¹ Division of Environmental Science and Ecological Engineering, Korea University,
Seoul 02841, Korea

² Graduate School of Bioresources, Mie University,
1577 Kurima-machiya, Tsu, 514-8507, Japan

³ Division of Forest Diseases and Insect Pests, Korea Forest Research Institute,
Seoul 02455, Korea

* CORRESPONDENCE TO: hdshin@korea.ac.kr

ABSTRACT—Conspecific Korean and Japanese powdery mildew specimens (including the type of *Sphaerotheca kusanoi*) on *Quercus* spp. previously identified as *Cystotheca lanestris* have been revealed as morphologically and phylogenetically distinct from North American *C. lanestris* specimens. The Asian specimens are confirmed as a separate species under the new combination *Cystotheca kusanoi*. *Cystotheca kusanoi* lacks distinct fibrosin bodies, even in fresh conidia and conidiophores. A key to *Cystotheca* spp. is provided.

KEY WORDS—*Cystothecaceae*, *Erysiphaceae*, *Erysiphales*, oaks

Introduction

Cystotheca (*Erysiphaceae*, tribe *Cystothecaceae*) is known to infect 62 host species, all except one belonging to *Fagaceae*. The genus is distinguished from other genera by the presence of special aerial hyphae (although these are absent in *C. esetacea* Z.X. Chen & Y.J. Yao) and the presence of two distinct layers in the chasmothecial wall (Braun & Cook 2012). In addition, conidia and conidiophores of *Cystotheca* species have been described as containing well-developed fibrosin bodies (Homma 1937, Braun 1987, Nomura 1997, Braun & Cook 2012).

Eight *Cystotheca* species have been recorded worldwide: *C. esetacea*, *C. indica* M.S. Patil & Maham., *C. lanestris* (Harkn.) Miyabe, *C. nanyuensis* (J.L. Zhou) U. Braun, *C. quercina* N. Ahmad & al., *C. tjobodensis* (Gäum.) Katum., *C. wrightii* Berk. & M.A. Curtis (Braun & Cook 2012), and *C. castanopsidis* (Meeboon & S. Takam.) Meeboon & S. Takam. (Wijayawardene & al. 2017). All species are native in Asia except for *C. lanestris*, originally described from North America but later recorded also from Asia.

Cystotheca lanestris is prevalent on various hosts that belong to the *Fagaceae* in North America and Asia (Braun & Cook 2012, Farr & Rossman 2018). Since 1940 in Korea, *C. lanestris* has been found on several *Quercus* species (Anonymous 1940; Amano 1986; Shin 1991, 2000). However, an investigation of Korean specimens revealed that the fungus occurring on *Quercus* species in Korea does not correspond with *C. lanestris* previously described from North America (Shin 1991). Further study using fresh samples collected throughout Korea led to the conclusion that the Korean specimens differ morphologically from all previously described species of *Cystotheca*. In addition, Japanese collections of *C. lanestris* s. lat. on *Quercus* spp. are morphologically similar to Korean collections. Here we report molecular sequence analyses of Korean and Japanese isolates from *Quercus* spp., to determine whether their phylogenetic placement agrees with their morphological similarity.

Materials & methods

Sample collection

Surveys were conducted at random at all sites in Korea, depending on accessibility at the time of the visits. Sites where diseases were observed were visited multiple times between 2011 and 2016 to obtain sufficient material for analyses. All collected plant material was processed on the day and place of collection. Representative specimens were deposited in the Herbarium of the Korea University, Seoul, Korea (KUS), and previously deposited *Cystotheca* species were re-examined. Japanese herbarium specimens labelled as "*Cystotheca lanestris*" were provided by the Tokyo University of Agriculture, Tokyo, Japan (TUAT; 11 specimens) and the National Museum of Nature and Science, Tsukuba, Japan (TNS; 24 specimens, including the isotype of *Sphaerotheca kusanoi*).

Morphological observation

The fungal fructification was detached from the infected leaves and mounted in a few drops of distilled water on a glass slide for light microscopy. Fungal structures were morphologically examined by bright field and differential interference contrast (DIC) light microscopy using an Olympus model BX51 microscope for measurements

and a Zeiss AX10 microscope equipped with a Zeiss AxioCam MRc5 for photography. The measurements were made at 100×, 200×, 400×, and 1000× magnifications.

DNA extraction, PCR amplification, and sequencing protocols

Genomic DNA was extracted from mycelia and chasmothecia using the Chelex 100 as previously described (Walsh & al. 1991, Hirata & Takamatsu 1996). The internal transcribed spacer (ITS) regions and large subunit (28S) ribosomal DNA were selected for the full survey. The ITS1+5.8S+ITS2 region was amplified using primers PMITS1 (5'-TCG-GAC-TGG-CCC-AGG-GAG-A-3') AND PMITS2 (5'-TCA-CTC-GCC-GTT-ACT-GAG-GT-3') (Cunnington & al. 2003), while domains D1 and D2 of 28S rDNA were amplified using primers PM3 (5'-GKG-CTY-TMC-GCG-TAG-T-3', Takamatsu & Kano 2001) and TW14 (5'-GCT-ATC-CTG-AGG-GAA-ACT-TC-3', Mori & al. 2000).

The genomic DNAs served as a template for polymerase chain reaction (PCR). The PCR mixture contained 12.5 µl of 2x PCR buffer for KOD FX Neo, 5 µl of 2 mM of each dNTP, 0.375 µl of 20 mM of each primer, 0.5 µl of KOD FX Neo polymerase, and 1 µl of template DNA. The PCR products were checked on 1.2% agarose gels and examined under ultra-violet light. Sizes of the PCR products were estimated by comparison with a TaKaRa Bio 1 kb molecular weight standard DNA ladder. The PCR products were then purified using the Qiagen QIAquick PCR Purification Kit. The products were bidirectionally sequenced by a commercial sequencing company (Macrogen, Seoul, Korea) with the same primers used for PCR.

Sequence alignment and phylogenetic analyses

All obtained sequences were edited using DNASTAR Lasergene SeqMan and Editseq software version 7.1.0 and MEGA version 7 (Kumar & al. 2016). Sequences obtained in this study were deposited in GenBank. Alignments were made with an online version of MAFFT7 (<http://mafft.cbrc.jp/alignment/server/>) (Katoh & Standley 2013). Phylogenetic trees were inferred from the maximum likelihood (ML) and maximum-parsimony (MP) methods in PAUP* 4.0, and Bayesian (BI) analyses in MRBAYES 3.1.2. ML analyses were performed using RAXML HPC BlackBox ver. 8.1.11 (Stamatakis 2014) using the default option with the generalized time reversible (GTR) substitution model implemented in the CIPRES cluster server (<https://www.phylo.org/>) at the San Diego Supercomputing Center. MP (Fitch 1971) analysis was carried out using PAUP* 4.0b10 (Swofford 2003). MP genealogies for a single gene were constructed using the heuristic search option with 100 random taxon additions and tree bisection and reconnection (TBR). Gaps were treated as missing data and all characters were unordered and of equal weight. Statistical support for nodes was obtained by performing 1000 bootstrap replicates. In addition, the consistency index (CI), retention index (RI), and tree length (TL) were determined for the resulting trees. ML inference was conducted using RAXML version 7.2.6 (Stamatakis 2014). The statistical selection of an evolutionary best-fit model of nucleotide substitution was carried out using jModelTest 0.1.1 and the Akaike's Information Criterion (AIC) (Darriba & al. 2012). The Bayesian inference (BI) analysis was performed for each individual data set based on a Markov chain Monte Carlo (MCMC) algorithm using

MrBayes version 3.2 (Ronquist & al. 2012). Two independent runs were performed simultaneously for 3 million generations every 100 generations. Burn-in values were determined with Tracer version 1.4 and the first 25% of the sampled trees representing the burn-in were discarded. Phylogenetic trees obtained from the BI and ML analyses were viewed in MEGA version 7 (Kumar & al. 2016).

Results

Morphology

The Korean and Japanese *Cystotheca* specimens on *Quercus* spp., including the isotype of *Sphaerotheca kusanoi*, were all morphologically similar but differed from *C. lanestris* from North America. We therefore propose a new combination for the Asian species:

Cystotheca kusanoi (Henn. & Shirai) S.E. Cho & H.D. Shin, **comb. nov.** FIGS 1, 2

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≡ *Sphaerotheca kusanoi* Henn. & Shirai, Bot. Jahrb. Syst. 29(1): 147, 1900 ["1901"].

TYPE: Japan, Prov. Kōzuke [Gunma Prefecture], Mt. Myōgi, on living leaves of *Quercus glandulifera* Blume [= *Q. serrata* Murray], 4 Nov. 1899, S. Kusano no. 123 (TNS-F-197792, isotype).

"*Cystotheca tenuis*" Miyabe & Takah., in Ideta, Pract. Phytopathol.: 170, 1901, nom. nud.

COLONIES hypophyllous, circular to irregular white patches, covering the whole lower leaf surface, at first whitish, later turning grayish to brown. SPECIAL AERIAL HYPHAE filiform, hyaline to pigmented, aseptate, 100–400 × 3.5–5 µm. APPRESSORIA indistinct or maybe absent. CONIDIOPHORES arising from the upper part of mother cells, 110–175 × 10–12.5 µm, producing 2–4 immature conidia in chains with a sinuate outline. FOOT-CELLS mostly straight or almost so, basal septum elevated 6–10 µm above junction with mother cells, 42.5–70 µm. PRIMARY CONIDIA conically rounded at the apex, subtruncate at the base. SECONDARY CONIDIA doliiform, limoniform, hyaline, 27–38 × 17–23 µm, with a length/width ratio of 1.4–1.8, without conspicuous fibrosin bodies. GERM TUBES very long, filiform, 0–1-septate, lateral to perihilar. CHASMOTHECIA hypophyllous, scattered or gregarious, partly embedded in special aerial hyphae, dark brown, spherical, 70–95 µm diameter, containing a single ascus, rarely two asci per chasmothecium. PERIDIUM consisting of two layers, outer wall composed of dark brown, polygonal cells, 10–20 µm wide. INNER WALL composed of

FIG. 1. *Cystotheca kusanoi*. A: Type collection (TNS-F-197792) of *Cystotheca kusanoi* in National Museum of Nature and Science, Tsukuba, Japan; B: Chasmothecium containing single ascus; C, D: Single ascus in an inner walled chasmothecium; E: Ascus containing 8 ascospores. Scale bars: B = 100 µm; C–E = 50 µm.

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CYPUS

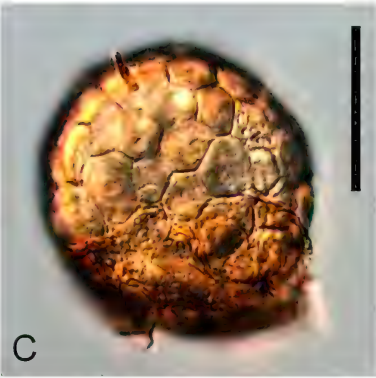
Sphaerotherca kusanoi P. Hennings et Shirai
on *Quercus glandulifera* Bl.

Miyougi

Coll. S. Kusano
No. F-197792

Date 4-XI-1899
Det.

A



hyaline, polygonal cells, 12.5–17.5 µm wide. APPENDAGES mycelioid, few, interwoven with the mycelium, pale brown to rusty brown, 4–6.5 µm wide. ASCI short-stalked, 80–110 × 57–65 µm, with a terminal oculus 12–15 µm wide, containing 8 ascospores. ASCOSPORES hyaline, oblong-elliptical, 23–30 × 16–19 µm.

SPECIMENS EXAMINED: KOREA, GANGWON, Gangneung, on *Q. variabilis*, 16 Jun. 1992, H.D. Shin (KUS-F11691; GenBank MG865450, MG865600); 22 Jun 1992, H.D. Shin (KUS-F11727; GenBank MG865451); 22 Jun 1992, H.D. Shin (KUS-F11729); 2 Aug 1992, H.D. Shin (KUS-F11827; GenBank MG865601); on *Quercus* sp., 1998, S. Takamatsu (VPRI 20287, GenBank AF073354); Goseong, on *Q. serrata*, 3 Sep 2012, H.D. Shin & S.E. Cho (KUS-F26873; GenBank MG865455, MG865606); on *Q. variabilis*, 3 Sep 2012, H.D. Shin & S.E. Cho (KUS-F26874; GenBank MG865456, MG865607); Samcheok, on *Q. variabilis*, 11 Oct 2009, H.D. Shin (KUS-F24728; GenBank MG865453, MG865603); 4 Sep 2012, H.D. Shin & S.E. Cho (KUS-F26885; GenBank MG865457, MG865608); 28 Aug 2011, H.D. Shin & S.E. Cho (KUS-F26041; GenBank MG865605); on *Q. serrata*, 15 Jun 2015, H.D. Shin & S.E. Cho (KUS-F28637; GenBank MG865459); on *Q. variabilis*, 15 Jun 2015, H.D. Shin & S.E. Cho (KUS-F28638; GenBank MG865460); 7 Sep 2015, H.D. Shin & S.E. Cho (KUS-F28817); on *Q. serrata*, 7 Sep 2015, H.D. Shin & S.E. Cho (KUS-F28818); on *Q. variabilis*, 7 Sep 2015, H.D. Shin & S.E. Cho (KUS-F28826; GenBank MG865461, MG865610); 20 Oct 2015, H.D. Shin & S.E. Cho (KUS-F28987; GenBank MG865462, MG865611); on *Q. serrata*, 20 Oct 2015, H.D. Shin & S.E. Cho (KUS-F28988, GenBank MG865463, MG865612); on *Q. variabilis*, 20 Oct 2015, H.D. Shin & S.E. Cho (KUS-F28989); Sokcho, on *Q. variabilis*, 13 Sep 2009, H.D. Shin (KUS-F24556); Yangyang, on *Q. serrata*, 26 Jul 2013, H.D. Shin & S.E. Cho (KUS-F27389; GenBank MG865458, MG865609); SOUTH GYEONGSANG, Namhae, on *Q. serrata*, 30 Jun 2004, H.D. Shin (KUS-F20369; GenBank MG865452, MG865602); NORTH JEOLLA, Gimje, on *Q. serrata*, 13 Jul 2010, H.D. Shin (KUS-F25062; GenBank MG865454, MG865604); JAPAN, FUKUSHIMA, 31 Oct 1953, Y. Nomura (TNS-F-128983); GUNMA, on *Q. glandulifera* [= *Q. serrata*], 4 Nov. 1899, S. Kusano no. 123 (TNS-F-197792, isotype); 22 Nov 1980, S. Tozuka (TUAT 1675); on *Q. mongolica*, 22 Nov 1980, S. Tozuka (TUAT 1739); ISHIKAWA, on *Q. mongolica*, 1 Aug 1973, S. Kakimoto (TUAT 131); on *Q. serrata*, 27 Sep 1980, S. Tanda & Yamazaki (TUAT 1286; GenBank MG865465, MG865614); 27 Sep 1980, Y. Nomura (TNS-F-129021, 129029); KANAGAWA, on *Q. serrata*, 6 Nov 1977, Y. Nomura (TNS-F-128986); NAGANO, on *Q. serrata*, 1 Oct 1987, S. Tanda & al. (TUAT 3882); on *Q. mongolica*, 22 Sep 1983, Y. Nomura (TNS-F-128991); on *Q. serrata*, 24 Sep 1983, S. Tanda & al. (TUAT 2196); 24 Sep 1983, Y. Nomura (TNS-F-129027); NIIGATA,

FIG. 2. *Cystotheca kusanoi*. A: Conidiophore: note the conically rounded apex of the uppermost immature primary conidium (prc); B: Conidiophore: note the subtruncate apex of the uppermost immature secondary conidium (sc); C: Conidia; a primary conidium is displayed in the upper left. Note the conically rounded apex (white arrow) and subtruncate base (black arrow), giving horizontal asymmetry; Other three conidia are secondary conidia with horizontal symmetry; D, E: Germinating conidia; F: Special aerial hypha; G: Chasmothecium containing single ascus; H: Chasmothecium containing two asci; I: Single ascus in an inner walled chasmothecium; J: Two asci in an inner walled chasmothecium; K: Ascus containing 8 ascospores. Scale bars: A–E = 30 µm; F, K = 50 µm; G–J = 100 µm.



on *Q. serrata*, 2 Nov 1979, S. Tanda (TUAT 1200); SENDAI, on *Q. mongolica*, 28 Sep 1973, Y. Nomura (TNS-F-128981); SHIZUOKA, on *Q. serrata*, 1 Oct 1973, Y. Nomura (TNS-F-128996); TOKYO, on *Q. serrata*, 20 Jul 1976, Y. Nomura (TNS-F-128994); 5 Sep 1976, Y. Nomura (TNS-F-128987, 129006); 19 Oct. 1980, S. Tanda & Yamazaki (TUAT 1351); YAMAGATA, on *Q. serrata*, 21 Oct 1977, S. Sato (TUAT 511; GenBank MG865464, MG865613); on *Q. mongolica*, 21 Oct 1977, S. Sato (TUAT 539); 28 Aug 1987, Y. Nomura (TNS-F-128982); YAMANASHI, on *Q. serrata*, 27 Oct 1957, Y. Nomura (TNS-F-128989, 129007); 3 Oct 1976, Y. Nomura (TNS-F-128990, 129025); 23 Oct 1977, Y. Nomura (TNS-F-128988); 6 Nov 1977, Y. Nomura (TNS-F-128997); 23 Oct 1979, Y. Nomura (TNS-F-129001); 1 Aug 1980, Y. Nomura (TNS-F-128993); 28 Oct 1980 Y. Nomura (TNS-F-128985); n.d., Y. Nomura (TNS-F-128928).

White mycelial mats were observed on the abaxial surface of the leaves, and the persistent mycelium later formed a dense brownish felt on the leaves.

Cystotheca lanestris is morphologically similar to *C. kusanoi* but differs by its longer (130–280 µm) conidiophores with longer (30–110 µm) foot-cells. The presence or absence of fibrosin bodies has not been described in detail in *Cystotheca*, and fibrosin bodies were not found in the fresh Korean specimens of *C. kusanoi*. Therefore, conidia and conidiophores should be examined in fresh *Cystotheca* specimens to confirm the existence of fibrosin bodies in the genus. The fibrosin lineage, including *Cystotheca*, *Podosphaera*, and *Sphaerotheca*, contains only a single ascus per chasmothecium (Braun 1987, Braun & Cook 2012). While Korean collections contained rarely two asci per chasmothecium, chasmothecia in Japanese collections consistently contained a single ascus.

Phylogeny

A BLASTn search with sequences retrieved from the Korean *Cystotheca* specimens against the NCBI nucleotide database showed that the Korean taxon is most closely related to *C. lanestris* (GenBank AB000933, AF011288, AF011289, MF170000). The phylogenetic trees constructed with the ITS and 28S rDNA region dataset supported the Korean and Japanese *Cystotheca* collections in an independent lineage that differed from *C. lanestris* collected in North America. In addition, the Asian species was supported as sister to *C. lanestris*, implying a close phylogenetic relationship between the two species, thereby confirming the phylogenetic position of *C. kusanoi* as a separate species in this study (Figs 3, 4). Although *C. kusanoi* is phylogenetically close to *C. lanestris*, DNA sequence comparisons based on the ITS and 28S rDNA gene regions clearly distinguish the two species, supported by strong bootstrap values, and endorse the hypothesis that they diverged from a common ancestor. GenBank AF073354 was recorded as *C. lanestris* on *Quercus* sp. from Korea (Cunnington & al. 2003), but

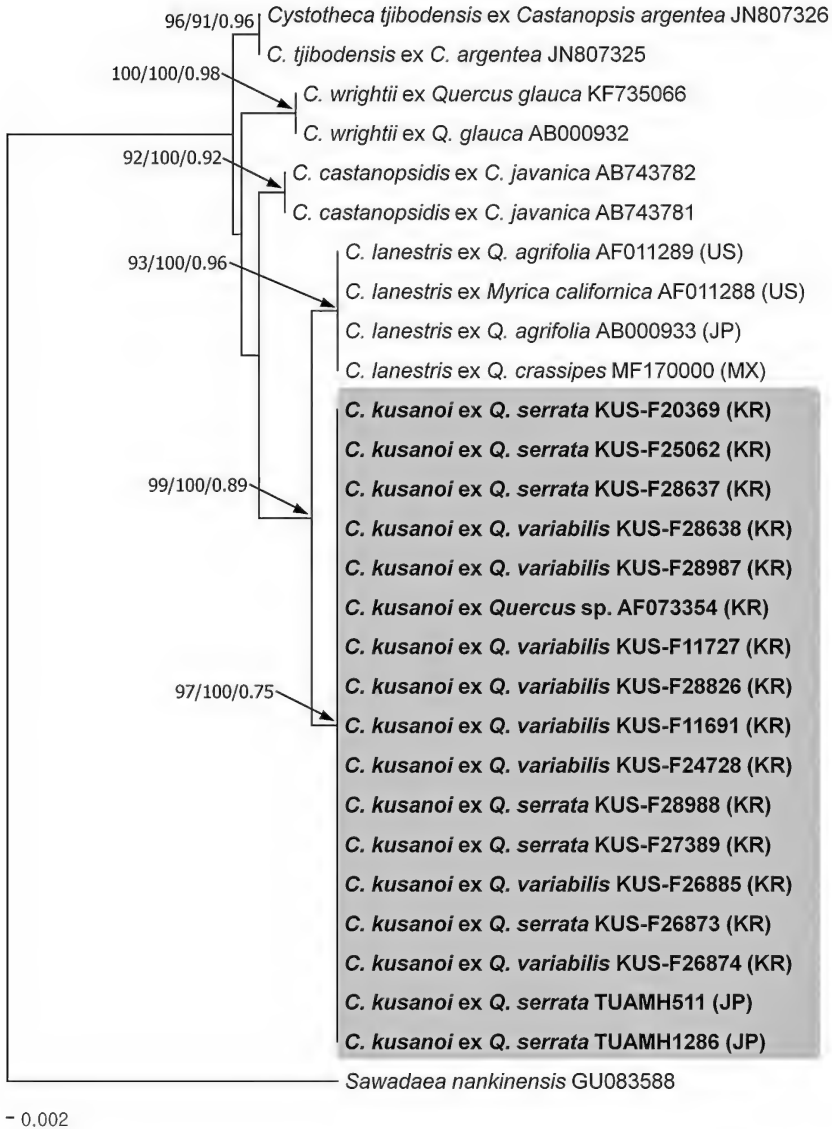


FIG. 3. Phylogenetic tree for *Cystotheca* inferred from maximum likelihood analysis based on ITS regions. MPBS, MLBS, and Bayesian PP above 50% are given above or below the branches. The number of nucleotide changes among taxa is represented by branch length, and the scale bar represents the number of nucleotide substitution per site.

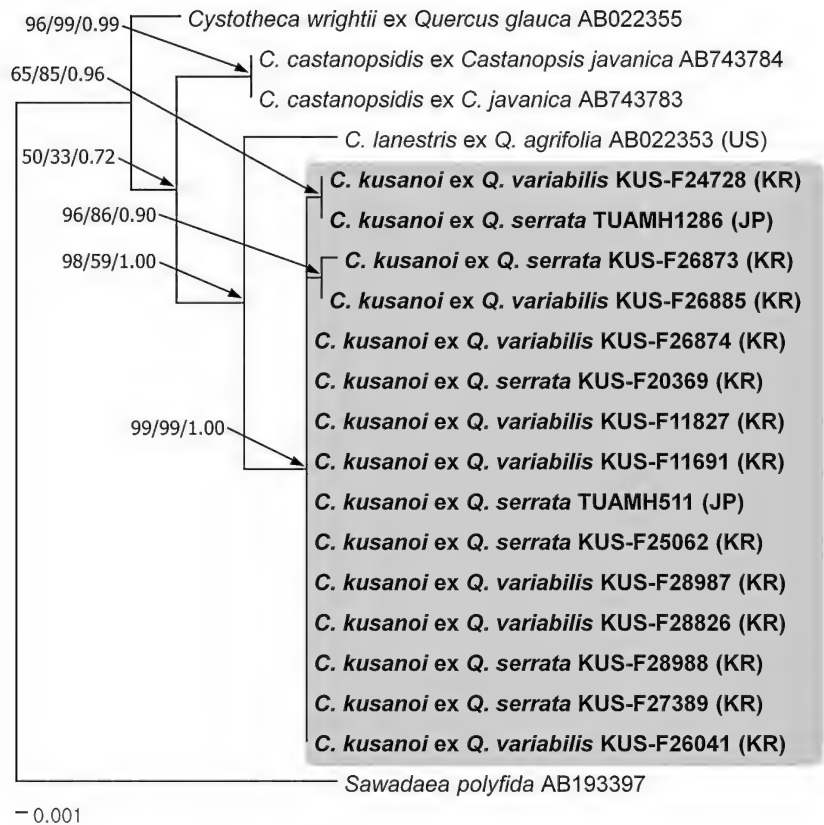


FIG. 4. Phylogenetic tree for *Cystotheca* inferred from maximum likelihood analysis based on the 28S region. MPBS, MLBS, and Bayesian PP above 30% are given above or below the branches. The number of nucleotide changes among taxa is represented by branch length, and the scale bar represents the number of nucleotide substitution per site.

our analyses place this specimen in the *C. kusanoi* clade while GenBank AB000933, derived from material collected at the University of California-Berkeley campus in 1995 by S. Takamatsu, is in the *C. lanestris* clade.

Discussion

Harkness (1884) described *Sphaerotheca lanestris* based on a Californian specimen found on *Quercus agrifolia* Née, an evergreen oak, and Salmon (1900) discussed and described in detail the species in his monograph of *Erysiphaceae*.

Hennings (1900) proposed the new species *S. kusanoi* for a specimen found on *Q. glandulifera* [= *Q. serrata*] collected in Japan by Kusano. Later, *Sphaerotheca lanestris* was transferred to the genus *Cystotheca* under the name *C. lanestris* and *S. kusanoi* was regarded as a synonym of *C. lanestris* (Ideta 1909).

Harkness (1884) described the anamorph of *Cystotheca lanestris* as:

“*Oidium ventricosum* Harkn.; segment swelling in the center and becoming barrel-shaped, $34\text{--}38 \times 20\text{--}22\ \mu\text{m}$, and filled with numerous round or elliptic bodies, $5\text{--}6 \times 2\text{--}4\ \mu\text{m}$, which are freely discharged from the end, as the joints separate.”

Sawada (1915) and Homma (1937) made similar observations. Meeboon & al. (2012) showed that *C. tjibodensis* has rather inconspicuous fibrosin bodies; and characterized *Cystotheca castanopsidis* as having rather inconspicuous fibrosin bodies (Meeboon & al. 2013, Wijayawardene & al. 2017). The description by Shin (1991, 2000) confirmed that powdery mildew species from Korea do not contain distinct fibrosin bodies in the conidial state. Furthermore, the descriptions and photographs of *Cystotheca wrightii* on *Quercus glauca* Thunb., recently reported from Korea by La & al. (2014), confirm that fibrosin bodies are also absent in the fresh conidia and conidiophores of that species. Thus, it appears that some *Cystotheca* species do not contain any conspicuous fibrosin bodies in their fresh conidia and conidiophores, and additional studies are needed to confirm these peculiarities in *Cystotheca*.

According to descriptions made by Shin (1991), the Korean *Cystotheca* specimens are similar to *C. lanestris* with regard to the symptoms produced and host species. However, Shin's (1991) examinations and observations in our study suggest that Korean *Cystotheca* specimens on *Quercus* spp. represent a separate species, clearly different from *C. lanestris* and which can be referred to as *C. kusanoi*, based on the previously described *Sphaerotheca kusanoi*. During extensive field forays searching for powdery mildew fungi, particularly for *Cystotheca* spp., *Q. serrata*, and *Q. variabilis* Blume were identified as Korean hosts of *C. kusanoi*. Since *Q. variabilis* has often been misidentified as “*Q. acutissima* Carruth.” in Korea, we cannot infer how wide the host range of the fungus might be in nature. In Japan, *Q. serrata* and *Q. mongolica* Fisch. ex Ledeb. were recorded as host species of *C. kusanoi*. The identity of other host plants remains unclear, and some collections on hosts recorded in the literature were unavailable for re-examination in Korea.

Our present morphological re-examinations and molecular analyses clearly support the new combination *C. kusanoi* for the powdery mildew fungus infecting *Quercus* spp. in Korea and Japan.

Key to *Cystotheca* species all known on *Fagaceae*

(except as otherwise annotated)

1. Mycelium amphigenous 2
1. Mycelium hypophyllous 3
2. Hyphal appressoria well developed, nipple-shaped *C. castanopsidis*
2. Hyphal appressoria lobed *C. quercina*
3. Special aerial hyphae absent *C. esetacea*
3. Special aerial hyphae present 4
4. Special aerial hyphae curved to falcate *C. wrightii*
4. Special aerial hyphae lacking or filiform 5
5. Chasmothecial appendages numerous, well developed, long;
non-fagaceous host *C. indica*
5. Chasmothecial appendages lacking or few, poorly developed, short 6
6. Mycelial patches dark brown or purplish brown 7
6. Mycelial patches whitish to light brown 8
7. Chasmothecia 90–130 µm diam. *C. tjibodensis*
7. Chasmothecia smaller, 60–70 µm diam. *C. nanyuensis*
8. Fresh conidia without distinct fibrosin bodies *C. kusanoi*
8. Fresh conidia with distinct fibrosin bodies *C. lanestris*

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First records of *Hydnobolites* and *Pachyphlodes* species from Turkey

YASIN UZUN & ABDULLAH KAYA*

Department of Biology, Science Faculty, Karamanoğlu Mehmetbey University,
Yunus Emre Campus, Karaman, Turkey

* CORRESPONDENCE TO: kayaabd@hotmail.com

ABSTRACT—The genera *Hydnobolites* and *Pachyphlodes* (*Pezizaceae*) are recorded from Turkey for the first time, based on collections of *Hydnobolites cerebriformis*, *Pachyphlodes citrina*, and *P. conglomerata*. Brief descriptions and photographs of the species are provided.

KEY WORDS—biodiversity, hypogeous ascomycete, truffle-like fungi

Introduction

Hydnobolites Tul. & C. Tul. and *Pachyphlodes* Zobel are two hypogeous ascomycete genera currently positioned in the family *Pezizaceae* Dumort. Kirk & al. (2008) and Healy & al. (2009a) report the existence of three described species of *Hydnobolites* and 12 described species of *Pachyphlodes*.

Species of *Hydnobolites* and *Pachyphlodes* are generally characterized by hypogeous and subglobose ascomata; ellipsoid to pyriform or broadly ellipsoid to clavate or cylindric, 8-spored asci; and globose, ornamented ascospores (Gilkey 1939, 1954; Hawker 1954; Pegler & al. 1993; Læssøe & Hansen 2007; Healy & al. 2009b; Trappe & al. 2009).

Thirty-nine members of *Pezizaceae* within *Adelphella* Pfister & al., *Iodophanus* Korf, *Marcelleina* Brumm. & al., *Pachyella* Boud., *Peziza* Dill. ex Fr., *Plicaria* Fuckel, *Sarcosphaera* Auersw., *Terfezia* (Tul. & C. Tul.)

Tul. & C. Tul. and *Tirmania* Chatin have so far been identified from Turkey. According to checklists (Sesli & Denchev 2014, Solak & al. 2015) and recently published data (Türkoğlu & al. 2015, Kaya & Uzun 2015, Elliot & al. 2016, Akata & Sesli 2017, Işık & Türkekul 2017, Sesli & Vizzini 2017, Türkekul 2017, Uzun & al. 2017a, b), no species of *Hydnobolites* and *Pachyphlodes* have previously been recorded from Turkey.

Here we report *Hydnobolites* and *Pachyphlodes* as the tenth and eleventh genera of Turkish *Pezizaceae* with the collections of *Hydnobolites cerebriformis*, *Pachyphlodes citrina*, and *P. conglomerata*. The study aims to contribute to the mycobiota of Turkey.

Materials & methods

Specimens were collected from Giresun, Rize, and Trabzon provinces in 2016 and 2017. Necessary ecological data were recorded, and the fruit bodies were photographed in the field. Macroscopic and microscopic measurements were performed in the laboratory. The material was mounted in Congo Red or Meltzer's reagent and observed microscopically using a Nikon Eclipse Ci-S trinocular light microscope. The specimens were identified with the help of Soehner (1935), Gilkey (1939), Vacek (1949), Hawker (1954), Lange (1956), Cazares & al. (1992), Pegler & al. (1993), Vidal (1997), and Hensel & Huth (2004). The specimens are preserved in the fungarium of Department of Biology, Science Faculty, Karamanoğlu Mehmetbey University, Karaman, Turkey (KMU).

Taxonomy

Hydnobolites cerebriformis Tul. & C. Tul.

Ann. Sci. Nat., Bot., Sér. 2, 19: 279 (1843)

FIG. 1

ASCOMA 6–30 mm in diameter, hypogeous, more or less globose, irregularly lobed or cerebriform, some with a small base formed by mycelial remnants mixed with substrate soil; surface glabrous, ochraceous yellowish to light brown. PERIDIUM with a pseudoparenchymatic structure of globose to isodiametric cells. GLEBA compact, concolorous with the surface or lighter. ODOR soft and pleasant. ASCI 80–130 × 65–90 µm, globose, thin-walled, generally 8-spored (some with 5–6 spores). ASCOSPORES 15–25 µm in diameter, spherical, thick-walled, hyaline and somewhat smooth at first, yellowish and reticulo-alveolate at maturity.

SPECIMENS EXAMINED—TURKEY, RIZE, Ardeşen, Eskiarmutluk village, 41°07'N 41°08'E, 610 m, mixed forest, in soil under *Corylus*, 5.08.2016, Y. Uzun 5182(KMU); TRABZON, Tonya, Sayraç village, 40°54'N–39°13'E, 1100 m, mixed forest, under *Fagus orientalis*, 8.11.2016, Y. Uzun 5415 (KMU).



FIG 1. *Hydnobolites cerebriformis* (KMU, Y. Uzun 5415).
a. Ascomata; b. Peridium; c,d. Asci and ascospores.

COMMENTS—Although Pegler & al. (1993) and Vidal (1997) reported the maximum size of ascomata as 13 and 10 mm, respectively, some of our samples reached almost to 30 mm. All the other morphological characteristics of the samples agree with the literature.

Pachyphlodes citrina (Berk. & Broome) Doweld,
Index Fungorum 31: 1 (2013)

FIG. 2

ASCOMA 10–30 mm in diameter, spherical, subglobose or irregularly lobed with an apical opening and a basal brown mycelial tuft, initially yellow to bright orange, later brown, dark brown to blackish, surface covered by 4–5 sided pyramidal warts. PERIDIUM pseudoparenchymatous. GLEBA compact, whitish with yellow veins when young, turning to bluish gray initially and in maturity or in contact with air to dark greenish gray; veins seem to be radiate from the base of the ascoma to the ostiole, some arising at the peridium and terminating after a short distance. ODOR soft and pleasant when young, becoming strong and unpleasant during the early decay stage. ASCI 120–160 × 38–45 µm, clavate to very broad, tapered below, stipulate, mostly 8-spored, spores biserial or arranged irregularly. ASCOSPORES 13–15 µm in diameter,



FIG 2. *Pachyphlodes citrina* (KMU, Y. Uzun 5152).
a–c. Ascomata; d. Peridium; e, f. Asci and ascospores.

spherical, covered with small 1.3–2 µm high sticks or truncated conical spines; first hyaline, then yellowish.

SPECIMENS EXAMINED—TURKEY, TRABZON, Tonya, Hoşarlı village, 40°56'N–39°18'E, in soil under *Quercus*, 740 m, 22.05.2016, Y. Uzun 5124; 20.06.2016, Y. Uzun 5152 (KMU); RIZE, Ardeşen, 41°10'N 41°06'E, 340 m, Ortaalan village, in soil under *Corylus*, 9.07.2017, Y. Uzun 5635 (KMU).

COMMENTS—The ascomata and habitat of our Turkish specimens generally conform to descriptions of *P. citrina* by previous mycologists (Soehner 1935, Gilkey 1939, Pegler & al. 1993, Vidal 1997). Our samples well fit with those reported by Gilkey (1939). Although truffles generally emerge upwards at maturity (Healy 2003), we collected most of our young fruit bodies close to the soil surface while obtaining our mature samples were at soil depths of 15–20 cm. When soaked in water, our dry samples smell like a boiled potato.

Pachyphlodes conglomerata (Berk. & Broome) Doweld

Index Fungorum 31: 1 (2013)

FIG. 3

ASCOMA 7–20 mm across, subglobose to irregular or lobed with an apical depression and a short hairy basal stalk, surface smooth or slightly tomentose



FIG 3. *Pachyphloides conglomerata* (KMU, Y. Uzun 5839).

a. Ascomata; b. Peridium; c,d. Asci and ascospores.

and yellow when young, then rough or wrinkled-lobed and brownish–yellow to (finally) blackish–brown. PERIDIUM pseudoparenchymatous, walls of outer cells coloured. GLEBA olive–yellow to gray when young, becoming dark gray to dark gray–brown at maturity; sparsely and indistinctly veined, veins whitish, but turning yellow in contact with air. ODOR not distinctive. ASCI 140–190 × 37–50 μm , cylindrical to clavate, rounded at the top, stipitate at the base, 8-spored, spores uniseriate, biseriate, or irregularly arranged. ASCOSPORES 15–18 μm in diameter, spherical, covered with 1.5–3 μm high, rod-like warts; hyaline when young, light brownish to greenish brown when mature.

SPECIMENS EXAMINED—TURKEY, GIRESUN, Dereli, Ağaçbaşı nature park, 40°42'N 38°16'E, 1170 m, in soil under *Fagus* and *Carpinus*, 13.09.2017, Y. Uzun 5822 (KMU); TRABZON, Sürmene, Ormanseven village, 40°51'N 40°08'E, 360 m, in soil under *Alnus*, *Castanea*, and *Rhododendron*, 14.09.2017, Y. Uzun 5839 (KMU).

COMMENTS—*Pachyphloides conglomerata* has been reported to occur under a number of deciduous and coniferous trees (Soehner 1935, Lange 1956), including *Fagus* and *Quercus* sp. (Vidal 1997). The morphology of our

samples agrees with previous descriptions, and their measurements also fall within the range given in literature. Soehner (1935), Vacek (1949), and Vidal (1997) reported maximum ascomatal sizes at or below 15 mm. Although some of our samples seem larger at 20 mm, they are not as large as the 15–30 mm specimens described by Hawker (1954).

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***Saksenamyces kamalii* gen. & sp. nov., from India**

JAISHRI SURYWANSHI^{1*}, AKHILA NAND RAI¹, PARAS NATH SINGH²

¹ Department of Botany, Dr. H.S. Gour Vishwavidyalaya, Sagar (M.P.), India, 470003

² National Fungal Culture Collection of India, Biodiversity and Palaeobiology Group,
MACS Agharkar Research Institute, GG Agarkar Road, Pune 411004, India.

* CORRESPONDENCE TO: jaishri.surywanshi10@gmail.com

ABSTRACT—A new genus and species, *Saksenamyces kamalii*, is described and illustrated. The fungus is characterized by undifferentiated conidiophores and monoblastic, bulbous conidiogenous cells that bear obclavate, whip like, rostrate, multiseptate, brown conidia.

KEY WORDS—foliicolous, phragmospore, *Shorea robusta*, taxonomic novelty

Introduction

Our current study is based on the morphological identification of novel fungus collected from Balaghat, Madhya Pradesh, India. During frequent periodic surveys conducted at a study site lying at 21°37'00"–22°18'30"N 80°02'30"–52°03"E, we encountered a conspicuous superficial black moldy symptoms on living leaves of *Shorea robusta*, an important timber-yielding plant in central India (Chitale & Behera, 2012). A detailed examination of the hyphomycete sporulating on the moldy areas determined that the unidentified fungus represented a new genus and species, proposed here as *Saksenamyces kamalii*.

Materials & methods

Frequent surveys were conducted in the Balaghat North Forest Division (November 2014) of Madhya Pradesh, India. During one field trip, the infected leaves of *Shorea robusta* were collected and brought to the lab for observation. Samples were mounted in lactophenol cotton blue and examined microscopically using a Leica DMLB LB30T light microscope and a FEI NOVA NANO SEM-450 Scanning Electron Microscope. Fifty measurements each of conidia and conidiogenous cells were recorded. The

holotype specimen is deposited in Ajrekar Mycological Herbarium, Agharkar Research Institute, Pune, India (AMH); and an isotype in the collections of Rai Jaishri Mycology Herbarium, Dr. Hari Singh Gour University, Sagar, India (RJM).

Taxonomy

Saksenamyces Surywanshi, A.N. Rai, P.N. Singh, **gen. nov.**

MYCOBANK MB 821284

Differs from the genus *Pseudocercospora* by its bulbous, monoblastic conidiogenous cells.

TYPE SPECIES: *Saksenamyces kamalii* Surywanshi & al.

ETYMOLOGY: The generic name honours and commemorates the services of the renowned, internationally acclaimed mycologist, the late Prof. S.B. Saksena from Sagar, M.P., India.

CONIDIOPHORES undifferentiated. CONIDIOGENOUS CELLS monoblastic, intercalary, bulbous to unequal subdoliiform, pale to olivaceous brown. CONIDIA solitary, obclavate, rostrate, phragmoseptate, sometimes unequally dichotomously branched, smooth, pale to olivaceous brown.

Saksenamyces kamalii Surywanshi, A.N. Rai, P.N. Singh, **sp. nov.**

FIGS 1–3

MYCOBANK MB 821285

Differs from *Pseudocercospora* species by its monoblastic, bulbous to unequal subdoliiform conidiogenous cells that are intercalary on undifferentiated conidiophores that resemble assimilative hyphae.

TYPE: On living leaves of *Shorea robusta* C.F. Gaertn. (*Dipterocarpaceae*) Baihar, North Balaghat Forest Division M.P., India, Nov. 2014 leg. J. Surywanshi, (**holotype**, AMH-9835; **isotype**, RJM 360).

ETYMOLOGY: refers to name of the renowned Indian mycologist from Gorakhpur, Prof. Kamal.

LEAF INFECTION amphigenous, predominantly hypogenous, superficial black moldy, irregular patches up to 1–17 mm in diameter. COLONIES amphiphylous predominantly hypophyllous, dark black, effuse, mycelium superficial, olivaceous brown, hyphae 1.5–4.5 µm thick, stromata present. CONIDIOPHORES undifferentiated, reduced into conidiogenous cells and resembling assimilative hyphae. CONIDIOGENOUS CELLS monoblastic, bulbous to unequal doliiform, thick-walled, intercalary, determinate, arising from the repent hyphae as a structure, pale to olivaceous brown, 6–19 × 4–9 µm. CONIDIA acrogenous, upright or prostrate, solitary, pale to olivaceous brown, long obclavate, whip-like, rostrate, straight to curved, occasionally unequally dichotomously branched, smooth, hila unthickened and stumpy, 1–24-phragmoseptate, 10–750 × 10.5–15 µm, 6–10 µm diam. at the base.

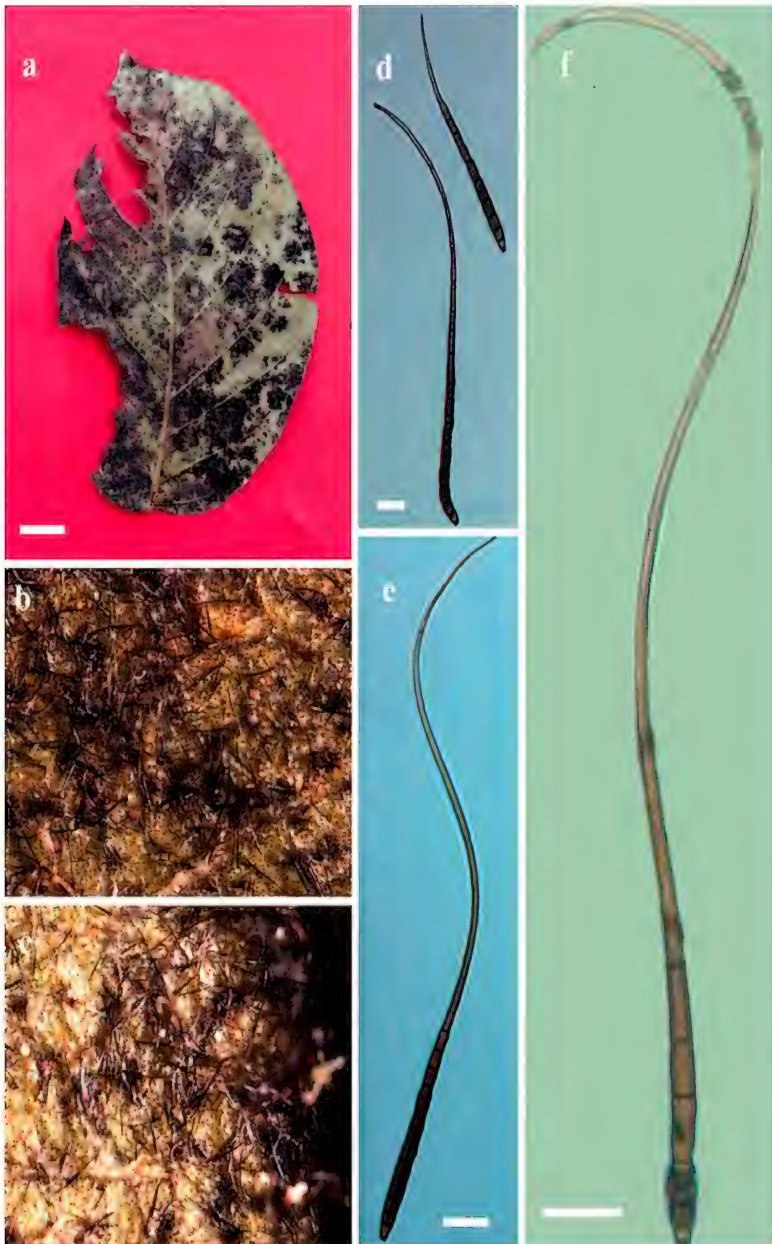


FIG. 1. *Saksenamyces kamalii* (holotype, AMH-9835). a. Lower leaf surface showing symptoms; b, c. Stereo microscope view of infected leaf showing numerous conidia; d–f. Rostrate and whip-like extremely long conidia. Scale bars: a = 2 cm; d–f = 20 μ m.

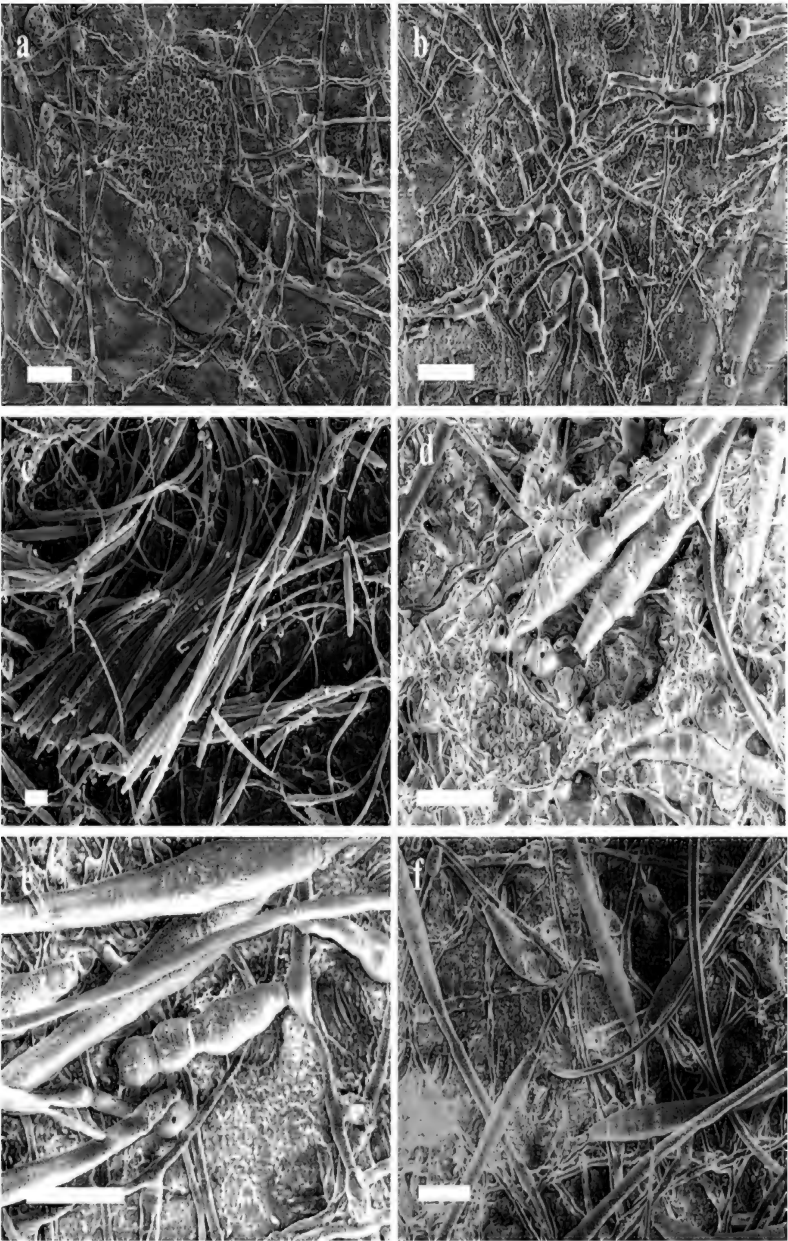


FIG. 2. *Saksenamyces kamalii* (holotype, AMH-9835). a. Mycelium arising from stroma; b. Host-fungus attachment; c. Bunch of conidia; d. Conidia showing conidiogenesis; e. Bulbous conidiogenous cell and attachment of conidium; f. Germinating conidia. Scale bars = 20 μm .



FIG. 3. *Saksenamyces kamalii* (holotype, AMH-9835). a. Stroma; b. Bulbous conidiogenous cells arising from non-differentiated conidiophore; c. Numerous conidia; d–e. Conidia straight to curved respectively; f–j. Conidia showing various types of germination. Scale bars: a–e = 20 μ m; f–j = 10 μ m.

REMARKS—*Saksenamyces kamalii* exhibits significant differences from allied fungal genera such as *Pseudocercospora* Speg., *Annellophora* Hughes, and *Clasterosporium* Schwein. and bears excessively long rostra, sometimes helicosporous in nature. The lack of differentiation makes the conidiophores unique. Despite successive efforts, we were unable to isolate and culture this new fungus.

Discussion

Saksenamyces kamalii is distinct from any other genus in having monoblastic, bulbous or unequal doliiform conidiogenous cells producing enormous conidia from lateral side and apical, long obclavate long rostrate conidia with some conidia bearing dichotomously branched rostra. *Saksenamyces* is superficially similar to *Pseudocercospora*, *Annellophora*, and *Clasterosporium* in producing obclavate conidia (Ellis 1971), but the conidiophores of these genera are differentiated, macronematous and the conidiogenous cells are terminal and nearly cylindrical. *Bulbocatenospora* (Castañeda-Ruiz & al. 2000) and *Chuppia* (Deighton 1965) have intercalary conidiogenous cells somewhat similar to those of *Saksenamyces kamalii* but differ by producing dictyospores (Seifert & al. 2011).

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***Mucor irregularis*, a first record for South America**

DIOGO XAVIER LIMA*, CARLOS A.F. DE SOUZA, RAFAEL J.V. DE OLIVEIRA,
JOSÉ LUIZ BEZERRA, ANDRÉ L.C.M. DE AZEVEDO SANTIAGO,
CRISTINA MARIA DE SOUZA-MOTTA

*Programa de Pós-graduação em Biologia de Fungos, Universidade Federal de Pernambuco,
Av. Prof. Nelson Chaves s/n, 50670-420, Recife, PE, Brazil.*

* CORRESPONDENCE TO: diogo_xavier00@hotmail.com

ABSTRACT—During a survey on the diversity of *Mucorales* in areas of Atlantic Forest and Caatinga (semiarid) in the state of Pernambuco, Brazil, two specimens of *Mucor irregularis* were isolated from soil and the roots of *Sorghum bicolor*. Their identity was confirmed by morphophysiology and ITS rDNA sequence analysis. The specimens are described and illustrated. This is the first record of *M. irregularis* in South America.

KEY WORDS—endophytic fungi; phylogenetic analysis, *Mucoromycotina*, taxonomy

Introduction

Mucor was erected by Fresenius in 1850 to include species that produce simple or branched sporangiophores that arise directly from the substrate, as well as globose or subglobose non-apophysate sporangia (Benny 2014). *Mucor* species are distributed around the world and are commonly found in soil and organic substrates, including stored grains, vegetables, and herbivore dung (Santiago & al. 2013, Lima & al. 2016). Several species of biotechnological importance are used in industrial processes, including the production of fermented foods (Abe & al. 2004, Millati & al. 2005). In contrast, *Mucor* taxa reported to cause human infection are regarded as among the most important clinical pathogens in *Mucorales* (Hoog & al. 2000, Ribes & al. 2000, Álvarez & al. 2009).

Mucor irregularis has been described as an emerging opportunistic fungus, especially in Asian countries. Its cutaneous or subcutaneous clinical infection affects faces and exposed extremities of immunocompromised patients, becoming chronic and leading to severe mutilation or patient death (Li & Lun 2012, Lu & al. 2013). Cases were initially reported in China, where *M. irregularis* (as *Rhizomucor variabilis* R.Y. Zheng & G.Q. Chen) was first isolated and described from a patient's lesions (Zheng & Chen 1991). Subsequent cases were reported from Australia (Ribeiro & al. 2010), Japan (Tomita & al. 2011), India (Hemashettar & al. 2011) and the USA (Schell & al. 2011). *Mucor irregularis* has also been isolated both from the soil and as an endophyte of *Rhizophora stylosa* Griff. (Lu & al. 2013, Gao & al. 2016). Nguyen & al. (2016) reported the isolation of *M. irregularis* from the gut of soldier fly larvae in Korea.

Mucor irregularis has been included in phylogenetic analyses by Álvarez & al. (2011), Walther & al. (2013), and Lu & al. (2013). Lu & al. (2013), who provided a detailed taxonomic and phylogenetic study based on four gene loci of *M. irregularis*. Focusing on geographic distribution, they reported that while *M. irregularis* is predominantly found in East Asia, it is distributed around the world. Its presence remains unconfirmed in Europe and South America, however.

The purpose of this manuscript is to describe and illustrate two isolates of *M. irregularis*, one from soil samples collected in the Atlantic Forest and the other as an endophyte from the roots of *Sorghum bicolor* (L.) Moench in crop systems in the semiarid region of Brazil.

Materials & methods

Soil and root collection

Soil samples were collected in the Saltinho Biological Reserve (8°43'09"S 35°10'11"W), located 100 km from Recife in the municipality of Tamandaré, Pernambuco, Brazil, which contains an area of Atlantic Forest. The climate is characterized as humid (As'; Köppen 1936) with a mean annual temperature of 25°C (Andrade & Moura 2011). Healthy roots of *Sorghum bicolor* were collected in crop areas of the Instituto Agrônomo de Pernambuco (the Pernambuco Agronomic Institute; IPA) in Serra Talhada, Pernambuco, Brazil (7°59'00"S 38°19'16"W). The climate of this region is characterized as semiarid (BSwh'; Köppen 1936) with a mean annual temperature of 25°C (Almeida & al. 2011).

Isolation of *M. irregularis* from the soil and from healthy roots of *Sorghum bicolor*

Five milligrams of soil were added to Petri dishes containing a wheat germ agar (WGA; Benny 2008) augmented with chloramphenicol (100 mg.L⁻¹). Root fragments

were cut and disinfected with 70% alcohol for 1 min, 2% sodium hypochlorite solution (NaOCl) for 2.5 min, and 70% alcohol for 30 s; after being washed twice with sterilized distilled water (Araújo & al. 2002), the root fragments were transferred in triplicate to Petri dishes containing malt extract agar (MEA; Benny 2008) plus chloramphenicol (50 mg.L⁻¹) to prevent bacterial growth.

The growth of colonies from the soil and roots was monitored for 96 hours at room temperature (26 ± 2°C) and subjected to alternate light and dark periods. In order to purify the *M. irregularis*, fragments of the mycelium were transferred separately to the malt extract agar medium (Benny 2008) augmented with chloramphenicol (80 mg.L⁻¹).

Morphological and physiological identification and mating experiments

Pure cultures from the isolates were cultured in triplicate on MEA and potato dextrose agar (PDA) and incubated at 15, 20, 25, 30, and 35°C for 15 days. Fragments of mycelia were removed from cultures, placed on microscope slides with KOH (3%), and observed under a Zeiss Axioscope 40 light microscope. The specimens were identified by observing their macroscopic (color, appearance, and diameter of colonies) and microscopic characteristics as described by Zheng & Chen (1991) and Lu & al. (2013). Colors of colonies were designated according to Maerz & Paul (1950). Mating experiments with the URM 7723 and URM 7724 strains were carried out on three MEA plates at 25°C. A 5-mm diameter disk was cut from each strain and placed on opposite sides of the plates, 3 cm apart. After three weeks the plates were checked for zygospores using a Leica EZ4 stereomicroscope with 95% ethanol added to each culture to facilitate observation.

Extraction and purification of rDNA

Fungal biomass was obtained from MEA cultures in test tubes stored at 28°C for up to 6 days and transferred to 2 mL microtubes with screw caps with 0.5 g of acid-washed glass beads with two different diameters (150–212 µm and 425–600 µm, 1:1; Sigma, USA). The material was crushed by stirring at high speed in a FastPrep homogenizer. Genomic DNA was extracted as described by Góes-Neto & al. (2005). The mycelium was washed with chloroform: isoamyl alcohol (24:1) and then homogenized in CTAB lysis buffer (2% cetyltrimethylammonium bromide, 20 mM EDTA, 0.1 M Tris-HCl pH 8.0, 1.4 M NaCl; Doyle & Doyle 1987, 1990). The DNA was precipitated in isopropanol, washed with 70% ethanol, and resuspended in 50 µL ultrapure water.

The primer pairs ITS1/ITS4 (White & al. 1990) were used to amplify the rDNA ITS1–5.8S–ITS2 region. The polymerase chain reaction was conducted as described by Oliveira & al. (2014). The final amplicons were purified with the Invitrogen™ PureLink™ PCR Purification Kit, sequenced directly or cloned with a CloneJet™ PCR Cloning Kit, in accordance with the manufacturer's instructions, and sequenced. The newly obtained sequences were deposited in GenBank.

Phylogenetic reconstructions were obtained by analyzing the ITS1–5.8S–ITS2 sequence data. The fungal sequences were aligned with Clustal X (Larkin & al. 2007)

and edited with BioEdit (Hall 1999). Prior to phylogenetic analysis, the optimal nucleotide substitution model was estimated using Topali 2.5. Bayesian inference analysis (two runs over 1×10^6 generations with a burn-in of 2500) was performed using MrBayes 3.1.2, and maximum likelihood analysis (with support estimated by bootstrap analysis with 1000 replicates) was performed using PhyML (Guindon & Gascuel 2003), launched from Topali 2.5.

Phylogenetic results

In BLASTn analysis, our new Brazilian sequences MG269827 and MG269828 had a 99% identity with *Mucor irregularis* JX976247 (CBS 700.71) and a 96% identity with *M. irregularis* JN206150 (CBS 103.93, the ex-type strain of *Rhizomucor variabilis*). Phylogenetic analysis of the sequence datasets (FIG. 1) showed that our fungal sequences MG269827 (URM 7723) and MG269828 (URM 7724) were grouped with the sequences of *M. irregularis*.

Taxonomy

Mucor irregularis Stchigel, Cano, Guarro & E. Álvarez,
Medical Mycol. 49: 71. 2011.

FIG. 2

≡ *Rhizomucor variabilis* R.Y. Zheng & G.Q. Chen, Mycosystema 4: 47. 1991;
non *Mucor variabilis* A.K. Sarbhoy 1965.

COLONY initially white, becoming yellowish to cream (MP 19D1) with yellowish reverse (MP 11J6), reaching 9.4 cm in diam. and 9 mm in height after 4 days in MEA at 25°C. SPORANGIOPHORES erect, sympodially and monopodially branched, arising from aerial hyphae (3–)5–15.5(–26.5) µm diam., hyaline. SPORANGIA globose to subglobose, (16–)28–40(–50) µm diam., grayish brown, with diffuent wall. COLUMELLAE globose 12.5–26.5 µm or ellipsoidal to cylindrical 30–33.5 × 22–30 µm, rarely conical 25–40 × 20–35 µm, some variable in shape, brownish, smooth-walled; collar small, little evident. SPORANGIOSPORES hyaline, smooth-walled, mostly ellipsoidal 2.5–11 × 2–4.8(–10), subspherical to ovoid 3.7–9.5 × 2.5–7 µm, or globose, 2.5–9.5 µm diam., some irregularly shaped and to 12 µm diam. RHIZOIDS observed. CHLAMYDOSPORES and OIDIA observed. ZYGOSPORANGIA not observed.

SPECIMENS EXAMINED: BRAZIL, PERNAMBUCO, Tamandaré, Saltinho Biological Reserve, 8°43'09"S 35°10'11"W, soil, 2016, D.X. Lima. (URM 7723; GenBank MG269827); Serra Talhada, IPA, 7°59'00"S 38°19'16"W, a healthy *Sorghum bicolor* root, 2016, R.J.V de Oliveira (URM 7724; GenBank MG269828).

HABITAT & DISTRIBUTION: Recorded from soil in an Atlantic Forest area in Pernambuco, northeastern Brazil and as endophytic in healthy roots of *Sorghum bicolor* in the crop systems in the semiarid region of Pernambuco.

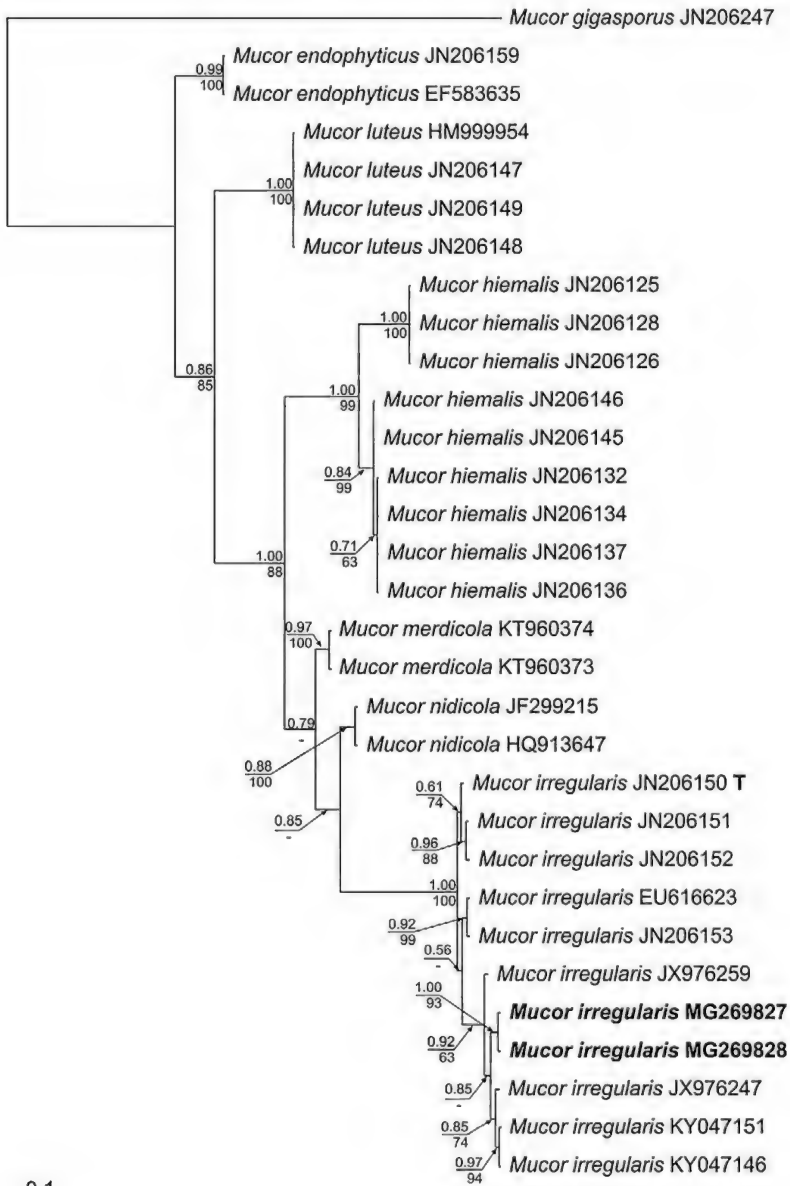


FIG. 1. Phylogenetic tree of *Mucor irregularis* and related species constructed using sequences of the ITS region. *Mucor gigasporus* was used as outgroup. Sequences are labeled with their database accession numbers. Support values are from Bayesian inference and maximum likelihood analysis. Our new Brazilian sequences are in boldface. Ex-type sequence of *M. irregularis* is annotated with T.

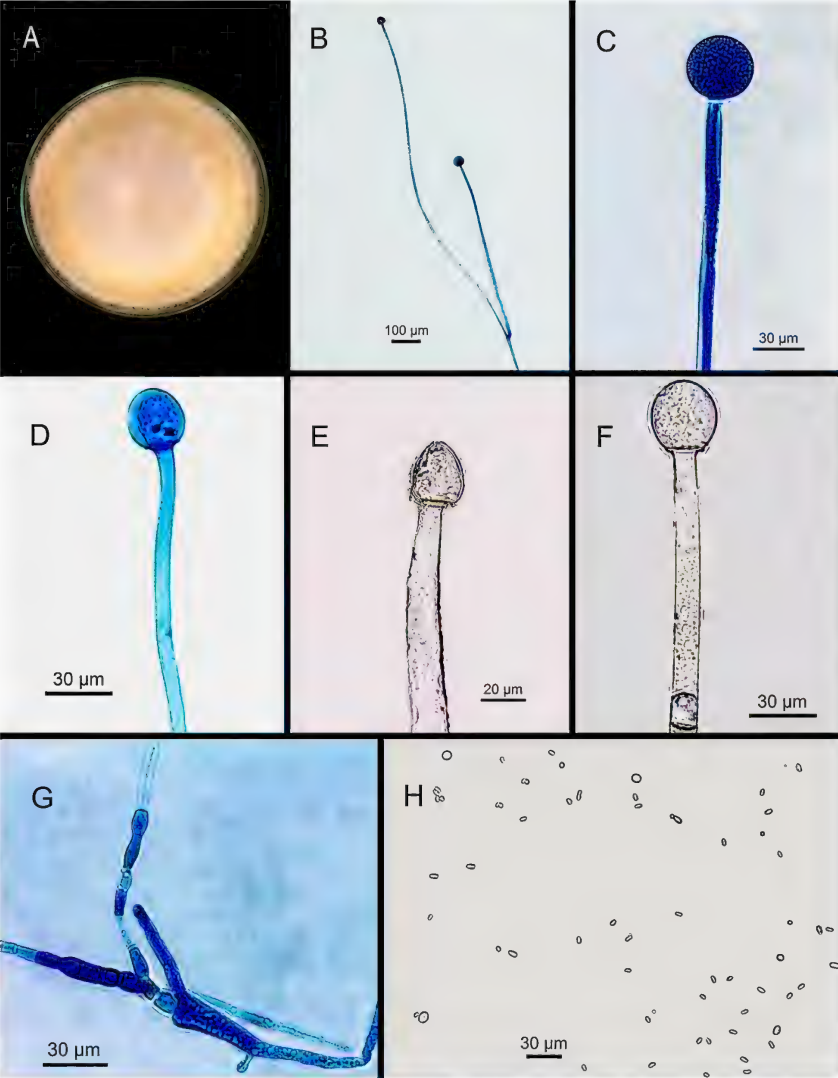


FIG. 2. *Mucor irregularis* (URM 7723): A. Colony; B. Sporangiphore with simple branch; C. Sporangiphore simple with sporangia; D. Sporangiphore simple with globose columella; E. Sporangiphore simple with conical columella; F. Sporangiphore simple with ellipsoidal to cylindrical columella; G. Chlamydozooids; H. Sporangiospores.

Previously reported from Australia (Ribeiro & al. 2010), China (Zheng & Chen 1991, Lu & al. 2009, Zhao & al. 2009, Li & Lun 2012), Japan (Tomita

& al. 2011), India (Hemashettar & al. 2011), Nigeria (Lu & al. 2013), South Korea (Nguyen & al. 2016), USA (Schell & al. 2011). This is the first report from South America.

Discussion

The morphological characteristics of *M. irregularis* described here show close similarity to the original description given by Zheng & Chen (1991, as *Rhizomucor variabilis*). However, we did not observe in our material the variably and irregularly shaped columellae cited in the original description; both Brazilian isolates exhibited globose and ellipsoidal to cylindrical columellae and mostly ellipsoidal sporangiospores, some of which were cylindrical or globose as reported by Lu & al. (2009, 2013) and Schell & al. (2011).

In the ITS rDNA phylogenetic tree (FIG. 1), *M. irregularis* sequences grouped closely with those of *M. hiemalis* Wehmer, which has morphological characteristics similar to *M. irregularis*. However, the sporangiophores of *M. hiemalis* are slightly sympodially branched, whereas *M. irregularis* produces monopodially and sympodially branched sporangiophores. The spherical to globose, grayish brown sporangia with diffluent walls of *M. irregularis* contrast to the globose, yellowish to dark brown sporangia with deliquescent walls observed in *M. hiemalis*. *Mucor irregularis* produces predominantly globose and ellipsoidal to cylindrical columellae that differ from the ellipsoidal with the truncate base type found in *M. hiemalis*. Additionally, *M. hiemalis* produces ellipsoidal sporangiospores (some flattened on one side) with few granules, whereas in *M. irregularis* the sporangiospores are smooth-walled, mostly ellipsoidal, cylindrical or globose (Schipper 1973).

Our Brazilian specimens, isolated from soil and as an endophyte in healthy roots, represent the first South American record of *M. irregularis*.

Acknowledgments

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FACEPE/CNPq, APQ, 0842-2.12/14). We also thank the Conselho Nacional de Desenvolvimento Científico e Tecnológico for the scholarship awarded to André L.C.M. de A. Santiago and Cristina M. de S. Motta. We thank Prof. Hyang Burm Lee and Dr Hsiao-Man Ho for the critical revision of the manuscript.

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Berberis lycium*: the newly discovered aecial host of *Puccinia striiformoides

Y. SOHAIL¹, B. ALI^{2,3}, K. GHAZAL², J. IQBAL²,
H. BADSHAH², A.S. MUMTAZ^{2*}

¹Government Degree College, Jhika Gali, Murree, 47150, Pakistan

²Department of Plant Sciences, Faculty of Biological Sciences, Quaid-i-Azam University,
Islamabad, 45320, Pakistan

³Department of Biochemistry, Genetics and Microbiology, Division of Genetics,
Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria,
Private Bag X20, Pretoria 0028, South Africa

*CORRESPONDENCE TO: asmumtaz@qau.edu.pk

ABSTRACT—*Berberis lycium* is reported for the first time as the alternate (aecial) host for *Puccinia striiformoides* from collections in Pakistan. This finding is confirmed by phylogenetic analyses of internal transcribed spacer (ITS) rDNA sequence data, which place our sequences of aecial *P. striiformoides* on *B. lycium* in a well-supported clade with GenBank sequences from uredinial *P. striiformoides* on *Dactylis glomerata*. Aecia of *Puccinia graminis*, *P. graminis* f.sp. *tritici*, and *P. brachypodii* that were also collected on *B. lycium* from Pakistan provide additional evidence of multiple rust species occurring on secondary host plant species.

KEY WORDS—*Berberidaceae*, *Pucciniales*, rust fungi, *Uredinales*

Introduction

About 400 species of rust fungi (*Pucciniales*) in 50 genera and five form genera on 350 plant species have been reported from Pakistan (Ahmad & al. 1997, Afshan & Khalid 2013). These include c. 80 species of rust fungi on ~93 host plants in the *Poaceae* recorded from Pakistan (Afshan & Khalid 2013).

Puccinia Pers. contains numerous species of heteroecious rust fungi with a life cycle that involves different spore stages on unrelated alternate hosts: uredinial, telial, and basidial stages on a primary host and pycnial (spermatial)

and aecial stages on a secondary host. *Berberis* spp. (barberries) have been reported as secondary hosts for several *Puccinia* species: *P. brachypodii* G.H. Otth, *P. brachypodii-phoenicoidis* Guyot & Malençon, *P. graminis* Pers., *P. montanensis* Ellis, *P. pseudostriiformis*, *P. pygmaea* Erikss., and *P. striiformis* Westend. (Berlin & al. 2013). Specifically, *B. lycium* has been reported as the alternate host for *P. brachypodii*, *P. graminis*, and *P. graminis* f.sp. *tritici* Erikss. & Henning (Staletić & al. 2004, Gautam & Avasthi 2016). Recently, *Berberis* sp. was revealed as an alternate host of *P. striiformis* through molecular barcoding of the internal transcribed spacer region (ITS) (Jin & al. 2010).

Berberis lycium is a medium-sized evergreen shrub widely distributed in northern Pakistan (Sood & al. 2012). It is used as food and in folk medicines. Many important phytochemicals have been identified in this species, which possesses antidiabetic, antihyperlipidemic, hepatoprotective, antibacterial, antifungal, anticoccidial, pesticidal, antimutagenic, and wound healing properties (Sher & Al- Yemeni 2011, Shabbir & al. 2012).

Phylogenetic analyses of the ITS region in combination with the large subunit (LSU) have been used to identify c. 200 *Puccinia* species (Jin & al. 2010, McTaggart & al. 2016a, Marin-Felix & al. 2017, Ali & al. 2017). However, almost no effort has been made to use phylogenetic methods to establish the linkages between aecial and uredinial/telial specimens with previously unknown heteroecious life-cycles (e.g., Padamsee & McKenzie 2017).

In the past, two varieties were known for yellow or stripe rust, *Puccinia striiformis*: *P. striiformis* var. *striiformis* and *P. striiformis* var. *dactylidis* (Cummins 1971). However, Abbasi & al. (2005) resolved the *P. striiformis* species complex into three distinct species using morphological and molecular approaches; their ITS sequence data resolved the three species as *P. striiformis*, *P. pseudostriiformis*, and *P. striiformoides*.

Puccinia striiformoides has been reported on *Poa pratensis* L. from Czech Republic and on *Dactylis glomerata* L. from Canada, Chile, China, Czech Republic, Hawaii, Iran, New Zealand, United Kingdom, and USA (Farr & Rossman 2017). *P. striiformoides* is also reported from Pakistan on *Lolium perenne* L. with uredinial and telial stages (Afshan & al. 2007). Here we present the first record of the aecial stage of *P. striiformoides* and the first record of *Berberis lycium* as an alternate host of this rust species.

Materials & methods

Rust infected *Berberis lycium* was collected during July–August 2014 from areas where barberry commonly grows at altitudes 1800–2400 m near the Punjab/Khyber Pakhtunkhwa border, Pakistan. Infected plants were found in regions with a

temperature range of 20–30°C during the day and 16–21°C at night, with an average humidity of 40–50%.

Berberis lycium leaves infected with aecia were collected and kept in newspaper and paper envelopes for preservation. After air-drying for three days, aecia were excised from a single local lesion and preserved at –80°C for later DNA extraction. *Triticum aestivum* plants infected with *Puccinia* (uredinia) were collected from Khyber Pakhtunkhwa, Punjab, and Sind during March 2014. The urediniospore samples of leaf rust were purified and multiplied under glass house conditions following Bariana & McIntosh (1994). The urediniospore isolates were preserved at –80°C for DNA extraction.

DNA was extracted from dried aecia and uredinia using a modified cetyltrimethylammonium bromide (CTAB) method (Ali & al. 2016). The extracted DNA samples were quantified in a NanoDrop™ 3300 Fluorospectrometer and 30 ng/μl was used for PCR amplification of ITS and 18S rDNA regions. The ITS region was amplified using ITS5/ITS4 primers (Justesen & al. 2003) and the SSU region using universal primers FG-F/FG-R (Einsele & al. 1997). The ITS and SSU PCR products of aecial and uredinial isolates were purified using Thermo Scientific™ GeneJET PCR purification kit according to the manufacturer's instructions and commercially sequenced by Macrogen in Korea. Raw sequence data were edited using BioEdit (Hall 1999), and sequences were deposited in GenBank.

The ITS sequences of aecial and uredinial isolates were BLASTed against the GenBank database (www.ncbi.nlm.nih.gov). A total of 34 ITS sequences for *Puccinia* species previously deposited in NCBI and the sequences generated in this study was used for the phylogenetic analysis. ITS sequences of aecial and uredinial isolates were aligned using Muscle E multiple alignment tool within MEGA v. 7.0 (Kumar & al. 2016). Phylogenetic analysis of the ITS sequences was performed using maximum likelihood (ML) method based on General Time Reversible Model (GTRMGAMMA; Nei & Kumar 2000). The percentage of trees in which the associated taxa cluster together is shown next to the branches (Fig. 1). Initial tree(s) for the heuristic search were obtained by applying the Neighbor-Joining method to a matrix of pairwise distances estimated using the maximum composite likelihood (MCL) approach. All positions containing gaps and missing data were eliminated. There were a total of 248 positions in the final dataset. Evolutionary analyses were conducted in MEGA 7.0 (Kumar & al. 2016). *Uromyces dactylidis* G.H. Otth was used as outgroup in phylogenetic analyses (Liu & Hambleton 2010).

Phylogenetic results

NCBI BLASTn analysis revealed that our ITS sequences matched closely with sequences of *P. striiformoides* in GenBank (Fig. 1). PAK-7 showed 99% identity with sequences from Canada (HM057110; 423/429 bp), China (HM057137; 337/340 bp), and South Africa (KP893702; 418/424); PAK-14 also showed 99% identity with sequences from Canada (HM057110;

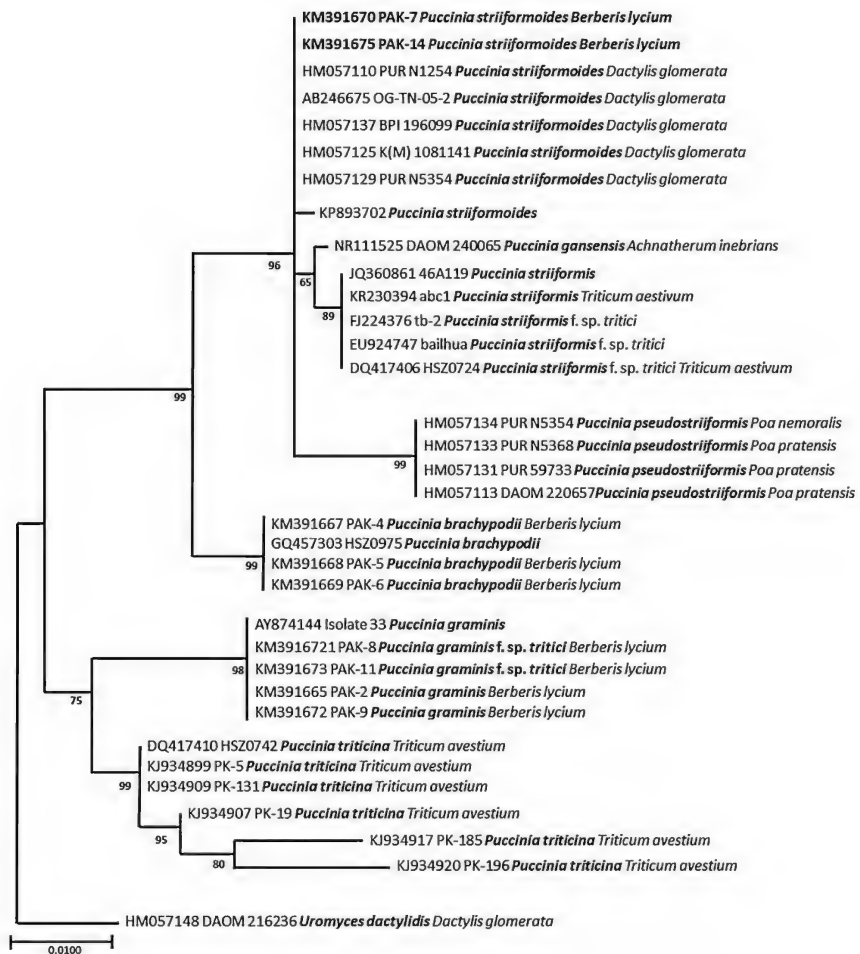


FIG. 1: A phylogram from maximum likelihood (ML) analyses based on ITS sequence data. The numbers on the branches are bootstrap values from ML analyses. Taxa in the phylogram are labeled as “accession number, strain or isolate, rust species name and host name”. The ITS sequences generated in this study are represented by PAK and PK numbers. *Uromyces dactylidis* was chosen as outgroup. Bootstrap values >65 are shown at the nodes.

423/429 bp), Japan (AB246675; 423/429 bp), and South Africa (KP893702; 418/424 bp). These BLAST results had highest identity to sequences such as HM057137 and HM057110 of *P. striiformoides* made by Liu & Hambleton (2010).

Taxonomy

Puccinia striiformoides M. Abbasi, Hedjar. & M. Scholler,

Rostaniha 5: 75. 2005 ["2004"].4)

PLATE 1

= *Puccinia striiformis* var. *dactylidis* Manners; Trans. Brit. Mycol. Soc. 43: 65. 1960.

= "*Puccinia striiformis* f.sp. *dactylidis*" H. Tollenaar, Phytopathology 57: 418. 1967.

PYCNIA (SPERMOGONIA) adaxial, 0.2–0.5 mm in diam., aggregated in small clusters; while PYCNIOSPORES are present, spots are yellowish to pinkish and surrounded by yellowish ring; after pycniospores have oozed out, spots become variously colored but usually pink to reddish surrounded by greenish ring. AECIA abaxial, yellowish, abundant, cylindrical, many individual aecia are aggregated into a single aecial sorus, aecidioid type with peridium, individual aecium raised; AECIOSPORES with yellowish contents, angular, elongated, subglobose or ellipsoid, verrucose, wall thickened, hyaline, $19\text{--}26 \times 16\text{--}22\ \mu\text{m}$.

MATERIAL EXAMINED —

Puccinia striiformoides aecia on *Berberis lycium* Royle: PAKISTAN, PUNJAB, Murree, Sunny Bank, altitude 2220 m asl, July 2014, leg. Y. Sohail PAK-14 (ISL-129547; GenBank KM391675, KP056210); KHYBER PAKHTUNKHWA, Ayubia, altitude 2400 m asl, July 2014, leg. Y. Sohail PAK-7 (ISL-129547; GenBank KM391670, KP056206).

Puccinia brachypodii aecia on *B. lycium*: PAKISTAN, PUNJAB, Murree, Aliyot, altitude 2200 m asl, July 2014, leg. Y. Sohail PAK-4 (ISL-129548; GenBank KM391667, KP056204); Barian, altitude 1798 m asl, August 2014, leg. Y. Sohail PAK-5,-6 (ISL-129548; GenBank KM391668, KM391669, KP056205).

Puccinia graminis aecia on *B. lycium*: PAKISTAN, PUNJAB, Murree, Mall road, altitude 2291 m asl, August 2014, leg. Y. Sohail PAK-2 (ISL-129549; GenBank KM391665); Patriata, Surasi, altitude 2285 m asl, August 2014, leg. Y. Sohail PAK-9 (ISL-129549; GenBank KM391672).

Puccinia graminis f.sp. *tritici* aecia on *B. lycium*: PAKISTAN, PUNJAB, Patriata, altitude 2285 m asl, August 2014, leg. Y. Sohail PAK-8 (ISL-129550; GenBank KM391671, KP056207); Ghora Gali, altitude 1900 m asl, August 2014, leg. Y. Sohail PAK-1 (ISL-129550; GenBank KM391673, KP056208).

Puccinia triticina on *T. aestivum*: PAKISTAN, PUNJAB, Okara, Chak 34 GD, altitude 105 m asl, March 2014, leg. Y. Sohail PK-19 (ISL-129551; GenBank KJ934907, KP056212); KHYBER PAKHTUNKHWA, Nowshara, altitude 404 m asl, March 2014, leg. Y. Sohail PK-131 (ISL-129551; GenBank KJ934909, KP056213); SIND, Matiari, Bypass, altitude 31 m asl, March 2014, leg. Y. Sohail PK-5 (ISL-129551; GenBank KJ934899, KP056211); Tando Allahyar, altitude 25 m asl, March 2014, leg. Y. Sohail PK-185 (ISL-129551; GenBank KJ934917, KP056214); altitude 25 m asl, March 2014, leg. Y. Sohail PK-196 (ISL-129551; GenBank KJ934920, KP056215).

Discussion

The present study established the aecial host of *P. striiformoides* as *Berberis lycium* using a molecular barcoding approach with the ITS region of rDNA.

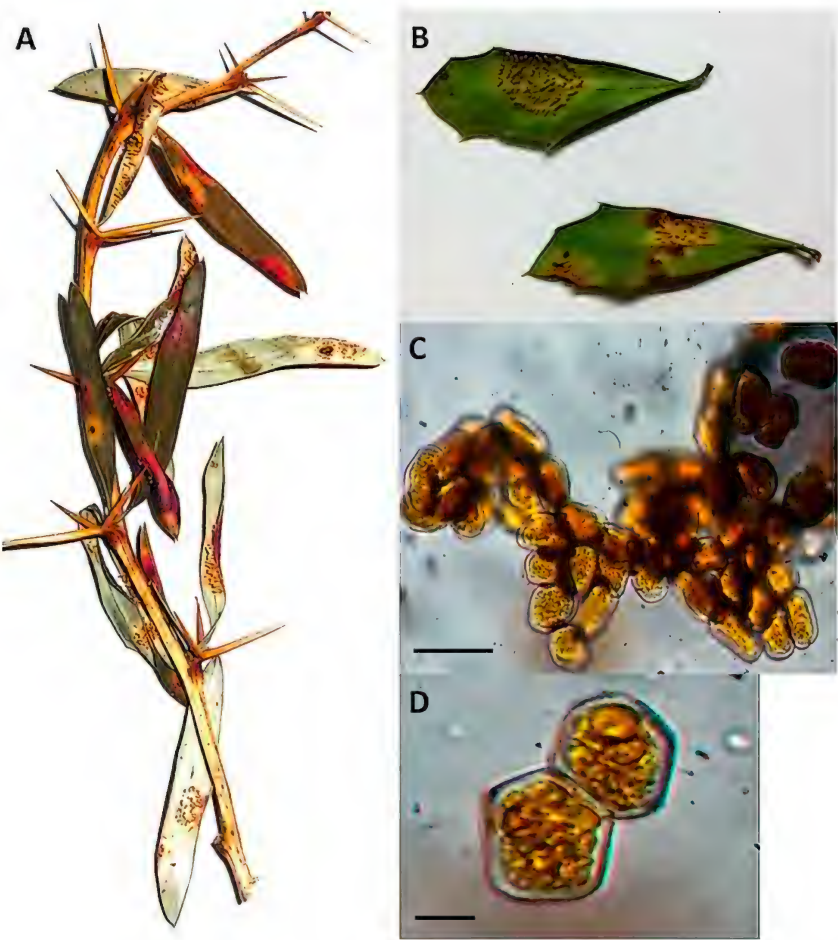


PLATE 1. *Puccinia striiformoides*. A. Aecial sori on abaxial surface of leaves of *Berberis lycium*, and old pycnial spots on the adaxial surface of leaves. B. Aecial pustules. C, D. Aeciospores. Scale bars: C = 10 µm; D = 5 µm.

These findings underscore that many aecial and telial stages of rust fungi are not yet linked, a continuing challenge due to the occurrence of aecia representing many different species on one host.

Species of *Berberis* are known as alternate hosts or aecial hosts for multiple *Puccinia* species including rusts on gramineous plants, especially wheat (*T. aestivum*), throughout the world (Cummins & Greene 1966, Urban 1967,

Jin & al. 2010). Previously, barberry was associated primarily with the aecial stage of wheat stem rust (*P. graminis* f.sp. *tritici*). Various *Berberis* species have now been identified as alternate hosts of *P. striiformis* (Zhao & al. 2013), the causal agent of wheat stripe rust. However, no previous study has assessed the possible role of *B. lycium* as an alternate host for other rusts.

BLASTn and phylogenetic analyses identified our aecial isolates from one host species as representing four rust species: *P. brachypodii*, *P. graminis*, *P. graminis* f.sp. *tritici*, and *P. striiformoides*. Our phylogenetic analysis clustered two of our isolates PAK-7 and PAK-14 with uredinial sequences of *P. striiformoides* from GenBank, which constituted a strongly supported monophyletic group. Aecial isolates PAK-7 and PAK-14 (collected from *B. lycium*, FIG. 1) represent the first aecial record of *P. striiformoides*. Previously Afshan & al. (2007) described the uredinial and telial stages of *P. striiformoides* on *Lolium perenne* in the Murree region, which clearly suggests that the pathogen exists in Murree on both primary and secondary hosts. The occurrence of *P. brachypodii*, *P. graminis*, *P. graminis* f.sp. *tritici*, and *P. striiformoides* on *B. lycium* shows the pathogen pressure on the natural stands of *B. lycium* and source of inoculum for the primary hosts in Pakistan. It is highly likely that their aecial stage has remained on barberry while the uredinial/telial stages have shifted hosts. McTaggart & al. (2016b) have recently linked such a host shift or subsequent jumps with shaping the diversity of rust fungi, which suggests further collection and investigation is needed, especially of primary hosts, to identify the diversity of *Puccinia* species in Pakistan and elsewhere in the world.

Furthermore, the investigation and establishment of unknown aecial stage with other rust spore stages is important. Aime & al. (2018), recently concluded that the aecial stage plays a role in the diversification of rust species with their hosts, which means the availability of aecial stages and related hosts will improve the systematics of rust fungi.

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New records of Brazilian hypogeous sequestrate fungi

N.M. ASSIS^{1*}, B.D.B. SILVA², I.G. BASEIA¹,
M.A. SULZBACHER³, M.P. MARTÍN⁴

¹ Departamento de Botânica e Zoologia, Centro de Biociências,
Universidade Federal do Rio Grande do Norte,
Avenida Senador Salgado Filho, 3000, Natal-RN 59.078-970 Brazil

² Departamento de Botânica, Instituto de Biologia, Universidade Federal da Bahia,
Ondina, Salvador, Bahia, 40170-115, Brazil

³ Departamento de Solos, Universidade Federal de Santa Maria,
CCR, Campus Universitário, 971050-900, Santa Maria, Rio Grande do Sul, Brazil

⁴ Departamento de Micología, Real Jardín Botánico,
RJB-CSIC, Plaza de Murillo 2. 28014 Madrid, Spain

* CORRESPONDENCE TO: nathalia.assis@outlook.com

ABSTRACT—Examination of specimens held in three Brazilian herbariums (UFRN, URM, ICN) for the genus *Rhizopogon* revealed that one collection represented *Alpova* cf. *australnicola*, a first record of the genus for Brazil. *Rhizopogon angustisepta* from South Brazil represents a new record for the Western Hemisphere; *R. verii* is a new record for Southeast and Northeast Brazil; *R. nigrescens* is tentatively reconfirmed from South Brazil, based on a poorly preserved specimen; and *R. marchii* is identified from a specimen with confused label information that does not indicate the country of origin.

KEY WORDS —Basidiomycota, Boletales, Paxillaceae, Rhizopogonaceae, taxonomy

Introduction

Species of *Rhizopogon* Fr., known as “false truffles,” have hypogeous and semi-hypogeous habits. The globose to subglobose basidiomata possess a simple structure: an external sterile peridium that protects the interior gleba, containing fertile chambers. The species are ectomycorrhizal, preferably with members of *Pinaceae* (Trappe 1962, Martín 1996, Grubisha & al. 2002), and distributed worldwide (Kirk & al. 2008).

Rhizopogon can be distinguished from other hypogeous sequestrate fungi by its lacunar gleba without columella and its smooth elliptical spores (Martín 1996); however, the genus has few reliably diagnostic morphological characters. Smith & Zeller (1966), using morphological characteristics and chemical tests (FeSO_4 , 5% KOH, and Melzer's reagent), described 121 new species for the genus. However, as discussed in Martín (1996) and Martín & al. (1998), chemical reactions together with peridium and gleba color generally distinguish only basidiome developmental stages and not distinctions between species. In general, morphological examination combined with molecular analyses is the preferable taxonomic approach for determining *Rhizopogon* species (Johannesson & Martin 1999). Smith & Zeller (1966) divided *Rhizopogon* into two subgenera: *R.* subg. *Rhizopogonella* A.H. Sm., based on whitish streaks observed in the gleba, and *R.* subg. *Rhizopogon*, based on the absence of these stretch marks. Trappe & Guzmán (1975), re-evaluating the species of *R.* subg. *Rhizopogonella*, regarded this subgenus as synonymous with *Alpova* C.W. Dodge, due to features of the hymenium and basidia.

Rhizopogon encompasses 150 species (Kirk & al. 2008). Although the genus is cosmopolitan, most species have been described from Pacific coastal coniferous forests of the United States of America (Grubisha & al. 2002) and on the European continent (Martín 1996). For Brazil, only eight species have been recorded, from the Southern and Southeastern regions and generally associated with introduced *Pinus* species: *R. fuscorubens* A.H. Sm., *R. luteolus* Fr., *R. nigrescens*, *R. roseolus* (Corda) Th. Fr., *R. rubescens* (Tul. & C. Tul.) Tul. & C. Tul., *R. verii*, *R. vulgaris* (Vittad.) M. Lange, and *R. zelleri* A.H. Sm. (Giachini & al. 2000, Baseia & Milanez 2002, Cortez & al. 2011, Sulzbacher & al. 2016). Index Fungorum (2018) and MycoBank (2018) list 26 species of *Alpova*, most described from the United States and Europe; they cite no records for *Alpova* in Brazil.

For this paper our intention was to increase the knowledge of *Alpova* (*Paxillaceae*) and *Rhizopogon* (*Rhizopogonaceae*) in Brazil by reviewing collections located in different Brazilian herbaria.

Materials & methods

Studied specimens are located in the herbaria of Universidade Federal do Rio Grande do Norte, Natal, Brazil (UFRN); Universidade Federal de Pernambuco, Recife, Brazil (URM); and Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil (ICN). Additional specimens from the Herbarium of Universidade Federal de Santa Catarina, Florianópolis, Brazil (FLOR) were lost in transit. Herbaria are abbreviated according to Thiers (2016). All descriptions are based on dried material.

Macroscopic studies follow methods in Martín (1996) and Baseia & al. (2014). We noted for each basidioma, the shape (globose, subglobose, and irregular), size, consistency and resistance of the mushroom to cutting, and the presence/absence of rhizomorphs. To determine color changes in the peridium and gleba, dried specimens were tested with standard reagents: 2.5% potassium hydroxide (KOH), 10% iron sulphate (FeSO_4), and Melzer's reagent (Largent & al. 1977). Colors were coded according to Kornerup & Wanscher (1978).

Microscopic observations followed Martín (1996); the four basic patterns of the peridium anatomy based on hyphal arrangement observed in *Rhizopogon* are: *luteolus*-type (hyphal packages arranged in different directions); *corsicus*-type (hyphal packages arranged in two different directions, intermixed or not with globose cells); *abietis*-type (hyphae forming a loosely interwoven weft); and *roseolus*-type (hyphae running parallel to the surface). We also recorded the presence of pigments and oleiferous hyphae in the peridium and gleba; size of the trama plates and diameter and arrangement of trama plate hyphae; subhymenium height; size and shape of basidioles, brachybasidioles, and basidia; and basidiospore size and shape.

All tissues were prepared with the aid of a Leica stereoscopic microscope and microstructures observed in a Nikon EclipsiNi optical microscope. The taxonomic concepts were based on specialized *Rhizopogon* literature and articles.

Results & discussion

One *Alpova* and four *Rhizopogon* species were identified and described (TAB. 1). *Alpova* cf. *austroalnicola* represents the first record of *Alpova* for Brazil, while *Rhizopogon angustisepta* represents the first record from the Western Hemisphere and *R. verii* the first record for Southeast and Northeast Brazil. *Rhizopogon nigrescens* is tentatively reconfirmed from South Brazil, based on a poorly preserved specimen (labeled as *R. cf. nigrescens*). *Rhizopogon marchii* is identified from a specimen with confused label information that does not indicate the country of origin.

TABLE 1. *Alpova* and *Rhizopogon* spp. identified in this study

SPECIES	HERBARIUM IDENTIFICATION	OCCURRENCE RECORD
<i>A. austroalnicola</i>	<i>R. luteolus</i> (UFRN-Fungos 144)	First record for Brazil
	<i>R. roseolus</i> (URM 10733)	
<i>R. angustisepta</i>	<i>R. roseolus</i> (ICN 154594; 168975)	First record for Western Hemisphere
	<i>R. provincialis</i> (URM 9403)	
<i>R. marchii</i>	<i>R. briardii</i> (URM 9468)	No location data
<i>R. cf. nigrescens</i>	<i>R. sp.</i> (UFRN-Fungos 2787)	Tentative second record for South Brazil
<i>R. verii</i>	<i>R. luteolus</i> (UFRN-Fungos 380; 1853)	First records for SE & NE Brazil
	<i>R. roseolus</i> (UFRN-Fungos 2786)	

Taxonomy

Alpova cf. *austroalnicola* L.S Domínguez, Mycologia 97: 599, 2005 FIG. 1A–C

BASIDIOMATA subglobose to irregular, $1.4\text{--}2.7 \times 0.9\text{--}2$ cm. PERIDIUM clay (5D5) to dark brown (6F5). RHIZOMORPHS covering all basidiomata, but some basidiomata are not covered by rhizomorphs on the upper surface. GLEBA dark brown (6F5) and firm in dried material.

MACROSCOPIC CHEMICAL REACTIONS—No color changes observed for dried peridium or gleba in KOH, FeSO_4 , and Melzer's.

PERIDIUM ≤ 395 μm high, hyphae < 4.5 μm diam., septate; in young material the peridium hyphal packets are arranged in different positions (*luteolus*-type), in mature specimens, forming a loosely interwoven weft similar to the *abietis*-type peridium. TRAMA PLATES < 66 μm , hyphae $4\text{--}5.1$ μm diam., hyaline, intertwined, refractive; some globose and subglobose cells between tramal plate hyphae, $14.8\text{--}24.7 \times 15\text{--}22.3$ μm . SPORES $6\text{--}7.8 \times 2.5\text{--}3.2$ μm , hyaline and ellipsoidal.

MATERIAL EXAMINED—BRAZIL. PERNAMBUCO: Cabo Santo Agostinho, Gurjaú Ecological Reserve, 24 March 2013, I.G. Baseia (UFRN–Fungos 144; as *R. luteolus*). URUGUAY. MONTEVIDEO: 15 May 1957, A.C. Batista & J.E Wright (URM 10733; as *R. roseolus*).

COMMENTS—The blackened gleba in the dried collections (FIG. 1A) is characteristic for *Alpova*. Nouhra & al. (2005) described *A. austroalnicola* from Argentina associated with *Alnus*. The Argentina species and our specimens shared similar trama plate and subhymenium cells, such as the globose cells in the gleba (FIG. 1B) and the basidiospore size and shape (FIG. 1C). However, the abundant dermocystidia noted for *A. austroalnicola* were not observed in our specimens. Furthermore, *A. austroalnicola* associates exclusively with *Alnus*, not found in Brazil, while our Brazilian specimen was collected in the native Brazilian Atlantic rainforest. There is

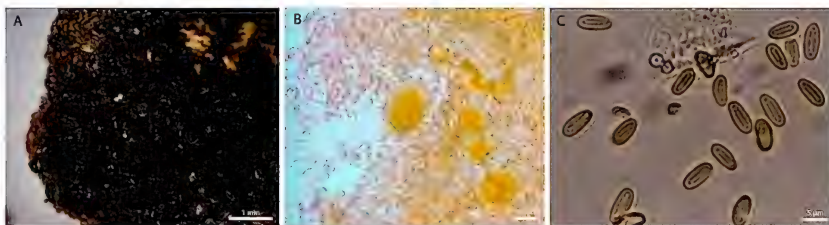


FIG. 1. *Alpova* cf. *austroalnicola* (UFRN–Fungos 144 as *R. luteolus*). A. Transverse section showing the peridium and gleba. B. globose and subglobose cells between trama plates. C. Spores.

no reference to the plant associate for the Uruguayan specimen. Our report represents the first records of *Alpova* for Brazil and Uruguay.

Rhizopogon angustisepta Zeller & C.W. Dodge,

Ann. Missouri Bot. Gard.5: 24, 1918

FIG. 2A–C

BASIDIOMATA subglobose to irregular 0.9–2 × 0.7–1.5 cm. Rigid fruiting body but crumbling away when cut. PERIDIUM dark brown (7F4) to reddish brown (6F5). Scarce rhizomorphs. GLEBA brownish brown (7E4).

MACROSCOPIC CHEMICAL REACTIONS—No color changes observed for dried peridium or gleba in KOH, FeSO₄, and Melzer's.

PERIDIUM *roseolus*-type, <300 µm high, hyphae brownish (Congo red), 7.7 µm (some reaching 12.6 µm) diam., arranged parallel to surface. TRAMA PLATES <46 µm broad, formed by interwoven hyphae, hyaline, refractive, branched and <5 µm diam. SUBHYMENIUM <28.1 µm diameter formed by subglobose (17.1–8.6 µm) and elongated (12.7–19.6 × 6–8.4 µm) cells. BRACHYBASIDIOLES clavate, 17.9–22.9 × 7–7.2, wall <2.2 µm diameter. BASIDIA cylindrical, 4.5–14.2 × 5.6–7.3 µm, thin walled (<0.5 µm); SPORES 7.0–10.5 × 3–4.5 µm, naviculate, biguttulate and with a basal scar.

MATERIAL EXAMINED—BRAZIL. RIO GRANDE DO SUL: Guaíba, Fazenda São Maximiano, 21 August 2010, L. Trierveiler-Pereira 93, under *Pinus* sp. (ICN 168975; as *R. roseolus*); Itaara, Pinhal Park, 21 April 2007, V.G. Cortez 069/07, under *Pinus* sp. (ICN 154594; as *R. roseolus*). PORTUGAL. SETÚBAL: 3 December 1957, C. Torrend (URM 9403; as *R. provincialis*).

COMMENTS—In *Rhizopogon angustisepta* neither the dried peridium nor the dried gleba changed color when tested with 5% KOH (FIG. 2A, B), whereas the dried peridium of *R. roseolus* changes to a reddish color. *Rhizopogon angustisepta* is characterized by naviculate spores (FIG. 2C). This species was known only from the type locality, Tilsit, East Prussia, Germany (now Sovetsk, Kaliningrad Oblast, Russian Federation), as indicated by Zeller & Dodge (1918), the only work on *Rhizopogon* included in GBIS (2016). Our newly identified specimens extend the distribution of *R. angustisepta* to western Europe, besides serving as the first record for to the Western Hemisphere.

Rhizopogon marchii (Bres.) Zeller & C.W. Dodge,

Ann. Missouri Bot. Gard. 16: 121, 1929

FIG. 2D–F

BASIDIOMATA subglobose to irregular, 11.5–13.1 mm. PERIDIUM brown leather (6E6), occasional blackish rhizomorphs present. GLEBA orange gray (5B4) to yellowish brown (5E8), crumbly, irregularly chambered.

MACROSCOPIC CHEMICAL REACTIONS—No color changes observed for dried peridium or gleba in KOH, FeSO₄, and Melzer's.

PERIDIUM *abietis*-type, <290 µm high. TRAMA PLATES <83 µm broad, hyphae septate, branched, hyaline, refractive, <4.5 µm diam. SUBHYMENIUM <33.8 µm diam.; cells 15–22 × 6.2–8 µm. BRACHYBASIDIOLES clavate, 12–16 × 7–6.5 µm. BASIDIA cylindrical 27.6–41.1 × 5.6–6.9 µm. SPORES 6.4–9.2 × 3.5–5 µm, smooth, hyaline, elongated.

MATERIAL EXAMINED—No location, 3 December 1957, J. Bresadola (URM 9468; as *R. briardii*).

COMMENTS—The basidiospores (FIG. 2F), subhymenial elements, and peridium type (FIG. 2E) support our identification of *R. marchii* for this collection, even though the extremely thin peridial layer (FIG. 2D) and the material conservation status made thorough microscopical observation difficult. The collection label provides very little reliable information: there are no location data, and 1957 is an unlikely collection date for the Italian collector(?), Bresadola (1847–1929). If this was collected by Bresadola, it is probably a European specimen, and the 1957 date may refer to its deposition in herb. URM. It seems more likely that the name of the collector is missing and that Bresadola's name was written (wrongly) as the authority of *R. briardii* Boud. We contacted URM curators to learn more about the origin of this material, but no new information was available.

***Rhizopogon cf. nigrescens* Coker & Couch,**

Gasteromycetes East. U.S: 30, 1928

FIG. 2G–I

BASIDIOMATA irregular, 2.1–1.6 cm diam. PERIDIUM grayish brown (9E2–8F2). GLEBA brown (6E4), irregular, labyrinthiform, firm, resistant to cutting.

MACROSCOPIC CHEMICAL REACTIONS—No color changes observed for dried peridium or gleba in KOH, FeSO₄, and Melzer's.

PERIDIUM <480 µm high, hyphae darkened, <3 µm diam. TRAMA PLATES <45 µm broad, hyphae hyaline, <3.5 µm diam. SUBHYMENIUM <30 µm diam., cells subglobose, 11–15.1 × 6.8–9.7 µm. SPORES 7.7–8.4 × 3–5.2 µm with truncate bases, biguttulate.

MATERIAL EXAMINED—BRAZIL. RIO GRANDE DO SUL: Dom Pedrito, 10 May 2009, M.A. Sulzbacher (UFRN-Fungos 2787; as *Rhizopogon* sp.).

COMMENTS—Material not preserved in good condition so that we were unable to determine the peridium type (FIG. 2G); only the trama plates

and spores were well observed (FIG. 2H, I). Giachini & al. (2000) recorded *R. nigrescens* from South Brazil, but the material requested from herb. FLOR was lost in transit.

Rhizopogon verii Pacioni, Bull. Soc. Myc. Fr. 100: 119, 1984

FIG. 2J–L

BASIDIOMATA subglobose to irregular 1–1.6 cm diameter. Peridium with fibrous brown surface (6E4), black at the base. Rhizomorphs brown (6E4) covering the entire surface of the peridium; under magnifying glass the wine-colored spots can be easily seen. Gleba firm when dry, bone hard, labyrinthiform, brown coloring (6F4) to grayish brown (6E3).

MACROSCOPIC CHEMICAL REACTIONS—No color changes observed for dried peridium or gleba in KOH, FeSO_4 , and Melzer's

PERIDIUM *luteolus*-type, <337 μm high, hyphae brownish in 5% KOH, prostrate, interwoven, 4–6 μm diam.; some hyphae had brown pigments, and were arranged in packets that then become loose. TRAMA PLATES hyphae interwoven, 2–5.1 μm diam., septate, branched. SUBHYMENIUM <20–23 μm broad, cells globose to subglobose, 12.9–17.8 $\times$ 7.5–11.2 μm . BRACHYBASIDIOLES clavate, 6–32 $\times$ 3–4.5 μm , thick-walled (<2.5 μm thick). BASIDIA lageniform, thick-walled (<1.5 μm thick); base ventricose, 17.8–24.7 $\times$ 4.2–5.6 μm ; beak thin-walled (<0.5 μm thick), 5–8.6 $\times$ 2–3 μm ; 4–8 spores developing in the apical portion. SPORES 5.7–8.4(–9.2) $\times$ 3.0–3.9 μm , ellipsoid, without visible guttule; some with a scar at the base.

MATERIAL EXAMINED—BRAZIL. SÃO PAULO: Instituto de Botânica, 24 April 1999, I.G. Baseia (UFRN-Fungos 380; as *R. luteolus*); RIO GRANDE DO SUL: Santa Maria, Centro de Educação Física, 21 May 2009, M.A. Sulzbacher (UFRN-Fungos 2786; as *R. roseolus*); CEARÁ: Tianguá, APA de Ibiapaba, 20 April 2012, D.S. Alfredo & B.T. Goto (UFRN-Fungos 1853; as *R. luteolus*).

COMMENTS—*Rhizopogon verii* is very close to *R. luteolus* but can be distinguished by the type of basidium: lageniform in *R. verii* (FIG. 2L) vs. cylindrical in *R. luteolus*. Pacioni (1984) considered *R. verii* close to *R. angustisepta*; however, in *R. angustisepta* the peridium is *roseolus*-type, and the spores are naviculate, while in *R. verii* the peridium is *luteolus*-type (FIG. 2J, K) and the spores are oblong-ellipsoid with a truncate base.

Rhizopogon verii was described from Tunisia (Pacioni 1984) and subsequently reported from Italy and Spain (Martín 1996). Sulzbacher & al. (2016) reported the first record for the Western Hemisphere from Rio Grande do Sul State, South Brazil. Our newly identified specimens extend the Brazilian distribution to São Paulo (Southeast Region) and Ceará (Northeast Region).

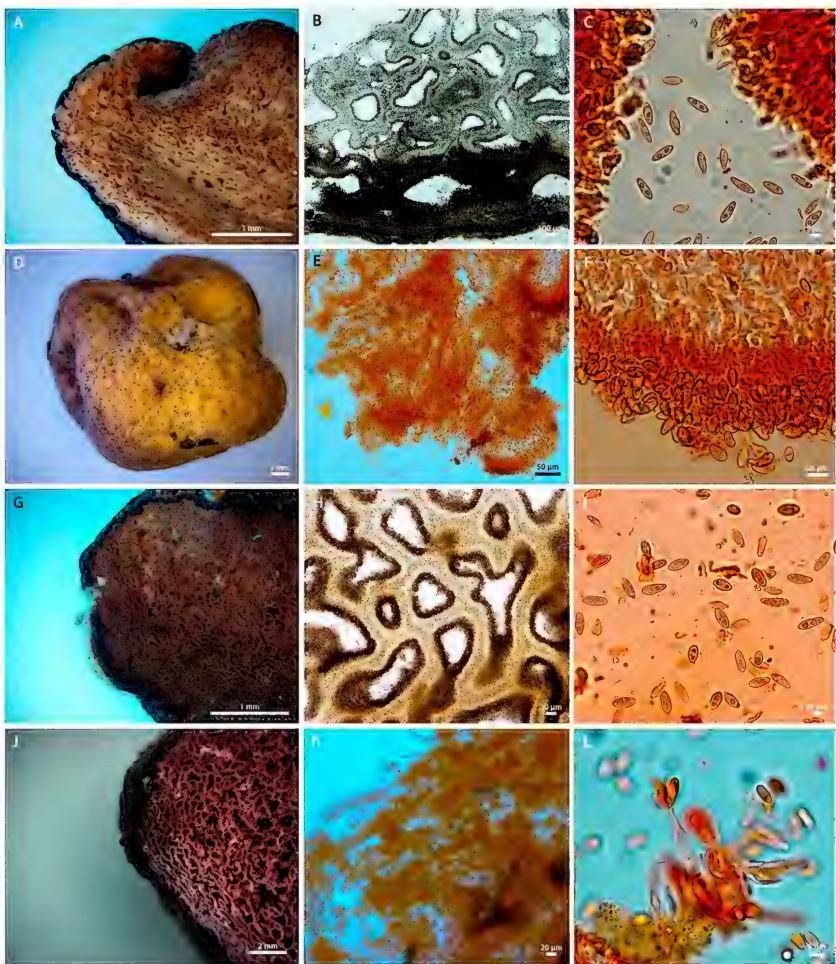


FIG. 2. Basidiomata and micromorphology of *Rhizopogon* species. *R. angustisepta* (URM 9403): A, B. Transverse section showing the peridium (darker region) and gleba (brighter region); C. Spores; *R. marchii* (URM 9468): D. Basidiomata; E. Peridium of *abietis*-type; F. Spores; *R. cf. nigrescens* (UFRN-Fungos 2787): G. Transverse section showing the peridium and gleba; H. Trama plates; I. Spores; *R. verii* (UFRN-Fungos 380): J. Transverse section showing the peridium and gleba; K. Peridium of *luteolus*-type; L. Lageniform basidia.

Discussion

All collections examined were initially misidentified, mainly as *R. luteolus* and *R. roseolus*. This is understandable because for a long time *Rhizopogon* species were identified based only on color changes in the peridium:

absence of change identified *R. luteolus*, while a change to red identified *R. roseolus*. However, as indicated in Martín (1996), other characteristics such as the arrangement of the peridial hyphae and the basidium type can help to distinguish species, even dried specimens in herbaria. Until now, it has not been possible for us to confirm the presence of *R. luteolus* or *R. roseolus* in Brazil, since all the material examined corresponds to other species.

If confirmed, *Alpova* cf. *austroalnicola* (also in *Boletales* but in a different family, *Paxillaceae*) was also initially labeled as representing *R. roseolus* or *R. luteolus*. Even in dried specimens, however, the gleba is a good diagnostic character to separate genera.

The importance of accurate herbarium labels became apparent when studying the specimen of *R. marchii*, since it was not possible to confirm the occurrence of this species in Brazil.

Our research has made it possible to expand the knowledge of taxonomy for *Alpova* and *Rhizopogon* as well as species distributions. However, many habitats have not yet been explored for hypogeous fungi, and new fieldwork will obviously further amplify the knowledge surrounding the taxonomic richness and species distribution of this group.

Acknowledgements

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***Tulostoma rufescens* sp. nov. from Sonora, Mexico**

EDUARDO HERNÁNDEZ-NAVARRO¹, ALDO GUTIÉRREZ¹,
JORGE H. RAMÍREZ-PRADO², FELIPE SÁNCHEZ-TEYER², MARTÍN ESQUEDA^{1*}

¹ Centro de Investigación en Alimentación y Desarrollo A.C.

Km 0.6 Carretera a La Victoria, Hermosillo, Sonora 83304, México

² Centro de Investigación Científica de Yucatán, A.C.

Calle 43 No. 130, Colonia Chuburná de Hidalgo, Mérida, Yucatán 97200, México

* CORRESPONDENCE TO: esqueda@ciad.mx

ABSTRACT—A new species of stalked puffball, *Tulostoma rufescens*, was observed and collected from subtropical scrub vegetation within the Sonoran Sky Islands, Mexico, and was characterized morphologically and molecularly. The new fungus is characterized by small to medium sized spore-sacs, a thinly membranous exoperidium persisting in patches in the pinkish endoperidium, a tortuous stem with a basal bulb strongly intermixed with sand and debris, subhyaline capillitia with swollen and pigmented septa, and strongly echinulate basidiospores with spines that occasionally coalesce to form a subreticulum. Maximum likelihood and neighbor-joining phylogenetic analyses of full ITS1-5.8S-ITS2 and D1-D2 LSU DNA regions placed our collection within the monophyletic genus *Tulostoma* but separate from all of the available sequenced species.

KEY WORDS—*Agaricaceae*, *Agaricales*, chorology, gasteroid fungi, molecular systematics

Introduction

The Madrean Sky Islands (or Madrean Archipelago) are a set of approximately 40 mountains in southern and southeastern Arizona, southwestern New Mexico, and northwestern Mexico. They combine temperate pine-oak forests at the highest elevations with arid to semiarid vegetation in the lowlands, the latter forming part of the Sonoran and Chihuahuan deserts. These environments preserve a remarkable richness, complexity, unusual neo- and archaeo-endemics, and an exceptional mixture of Nearctic and Neotropical

species (Warshall 1995). The biodiversity of the Madrean Sky Islands has been studied extensively, totaling close to two thousand species including 1380 plants, 358 birds, 104 reptiles and amphibians, 76 mammals, and 39 fish species (Van Devender & al. 2013), but information on fungal diversity is scant.

Sonora is the second largest Mexican state (179,355 km²) and ranks fifth of the 32 states of Mexico in fungal diversity with more than 618 morphospecies (Aguirre-Acosta & al. 2014), of which around 210 represent gasteroid and sequestrate *Agaricomycetes* (GSA), with *Tulostoma* Pers. (with 30 spp.) being the most diverse and representative genus (Hernández-Navarro & al. 2017). Some Sonoran GSA species, including the stalked puffballs, are rare worldwide; e.g., *T. portoricense* is known only from its type locality (Puerto Rico) and Sonora (Esqueda-Valle & al. 1998), and *T. gracilipes* only from its type locality (South Africa) and Sonora (Piña & al. 2010).

Tulostoma was proposed and sanctioned by Persoon (1794, 1801) and is characterized by gastrocarps composed of a hollow stalk inserted in a socket of a spore-sac with an apical ostiole. The genus has a cosmopolitan distribution but is especially richly diverse and abundant in arid and semiarid areas. According to Wright (1987), most species exhibit a terrestrial habit, with the remarkable exception of *T. exasperatum*, which grows in decaying wood. *Tulostoma* species can be classified based on their habitat as psammophilous (sandy soils in arid regions), terricolous (clay-loving species, in pastures, roadsides), and “forest soil-loving species” (in tropical or temperate zones with a high content of organic matter). In his *Tulostoma* world monograph, Wright (1987) included 139 species; at the present time, Species Fungorum accepts c 155 taxa (www.speciesfungorum.org).

There is scant molecular information on *Tulostoma* species. From the Americas, only *T. domingueziae* from Argentina has been described based on molecular data (Hernández Caffot & al. 2011); and from Asia only *T. ahmadii* from Pakistan (Hussain & al. 2016). Jeppson & al. (2017) made a major contribution, sequencing European species of *Tulostoma*, including 34 holotypes and some specimens from other continents. As a result, they characterized 30 known species, proposed five new species, and identified at least 27 new undescribed species; they also confirmed *Tulostoma* as monophyletic, found an unexpectedly large cryptic diversity, and cited areas of steppe vegetation in Hungary and Spain as hot spots for *Tulostoma* species diversity. The complete ITS1-5.8S-ITS2 nrDNA region is considered as the barcode for fungal species recognition. To reconstruct a better phylogeny, it has been suggested to complement this information with other ribosomal regions

such as D1-D2 LSU, and/or protein coding genes such as *atp6*, *EF1- α* , or *RPB1*, among others (Stielow & al. 2015).

As part of a major research, we studied some unidentified *Tulostoma* collections from the Sonoran Sky Islands whose morphological characterization did not match with any of the currently known morphospecies; their molecular characterization led us to propose *T. rufescens* as a new species.

Materials & methods

The studied material is preserved in the Collection of Macromycetes of Sonoran State University, Hermosillo, Mexico (UES). It was collected in sandy soil, in a subtropical scrub vegetation, located at 29°53 44 N 109°27 21 W at 1214 m asl. Basidiomes were characterized and conserved following conventional mycological techniques. Codes in parentheses after colors in basidiomata descriptions follow Kornerup & Wanscher (1978). Microscopic features were measured by examining gleba sections mounted in 10% KOH preparations using an Olympus BX-51 light microscope (LM). Fifty spores from each basidiome (including ornamentation, capillitia, and septa) were randomly measured and the mean and standard deviation were calculated. All measurements were made using the Infinity analyze Software v. 6.5.4 (Lumenera Corp.). Scanning electron microscope (SEM) micrographs were produced with a JEOL-JSM 600 LB microscope using critical point drying and sputtered with gold-palladium according to Moreno & al. (1995).

Genomic DNA was extracted following a standard CTAB 2% protocol (Cubero & al. 1999) with some modifications. After first grinding stipe tissue in liquid nitrogen and placing ~100 mg of dusted tissue into a 2 mL tube, 1 mL CTAB 2% + 100 μ L of β -mercaptoethanol were added, and the tubes were incubated at 55°C for 30 min, mixing every 10 min. Then, 600 μ L of chloroform : isoamylc alcohol (24:1) was added and mixed by inversion for 10 min. The mix was then centrifuged for 15 min at 12,000 $\times$ g; the supernatant was transferred to a 1.5 mL tube and 500 μ L of isopropanol + 50 μ L of 3M sodium acetate was added, mixed by inversion, and stored at -20°C for 1 h. The mix was centrifuged 10 min at 12,000 $\times$ g and the supernatant discarded. The remaining pellet was washed twice with EtOH 70%, dried at room temperature, and resuspended in 50 μ L of ultrapure water. The gDNA was then treated with 1 μ L of 10 mg/mL of RNase and stored for 30 min at 37°C, quantified in a NanoDrop™ 2000, and its integrity verified by visualization on a 1% agarose gel stained with Ethidium Bromide (EtBr).

The gDNA was diluted to 10 ng/ μ L to amplify nuclear ribosomal RNA genes (the full ITS1-5.8S-ITS2 and D1-D2 LSU regions); for this, we used the primer pairs ITS1/ITS4 and LR0R/LR5. PCR reactions were carried out in a volume of 20 μ L, with 20 ng gDNA, using the mix content and thermal cycler conditions described by Schoch & al. (2012). PCR amplicons were then visualized in a 1% agarose gel stained with EtBr. Amplicons were purified from the gel using the kit Wizard® SV Gel and PCR Clean-Up System and cloned using pGEM®-T-Easy following the manufacturer's instructions

using *Escherichia coli* DB10B chemically competent cells. The recombinant clones were grown in LB medium with IPTG (100 mM), ampicillin (50 µg/mL), and X-gal (50 mg/mL) as selection markers. The positive colonies were grown overnight in LB broth with ampicillin (50 µg/mL) and the pDNA was extracted using the alkaline lysis method (Sambrook & al. 1989). The presence of the inserts was verified by visualizing EcoRI enzymatic digestion of 1 µg of the pDNA, and also by PCR using the primers M13F and M13R in 1% agarose gels stained with EtBr. The pDNA was sequenced in triplicate by Macrogen Korea.

The obtained sequences were manually curated by inspecting their chromatograms on the SequencherSoftware™ v. 5.2.3 and the clean assembled sequence was used for BLASTN query at NCBI's GenBank. Then, a MegaBlast was performed, and 95 highly similar *Tulostoma* sequences and two outgroup sequences were downloaded from GenBank, aligned using the MUSCLE algorithm with default parameters (Edgar 2004), and manually edited using MEGA 6.0 software suite (Tamura & al. 2013). We performed two different molecular phylogenetic analyses for the 98 complete ITS1-5.8S-ITS2 and D1-D2 LSU rDNA gene sequences. One tree consisted of a Maximum likelihood (ML) phylogenetic analysis, with the GTR+G+I model (Nei & Kumar 2000) with gaps treated as partial deletions with a 95% of coverage, using an NNI heuristic method for topology improvement; the other consisted of a Neighbour-Joining (NJ) distance analysis, with gaps treated as pairwise deletions (Saitou & Nei 1987); both with 1000 bootstrap replicates. The trees were rooted using *Mycenastrum corium* as ingroup for *Agaricaceae* and *Psathyrella secotioides* as outgroup for *Psathyrellaceae* (Matheny & al. 2006, Larsson & Jeppson 2008, Moreno & al. 2015).

Results

Tulostoma rufescens Hern.-Nav. & Esqueda, sp. nov.

FIGS 1–6

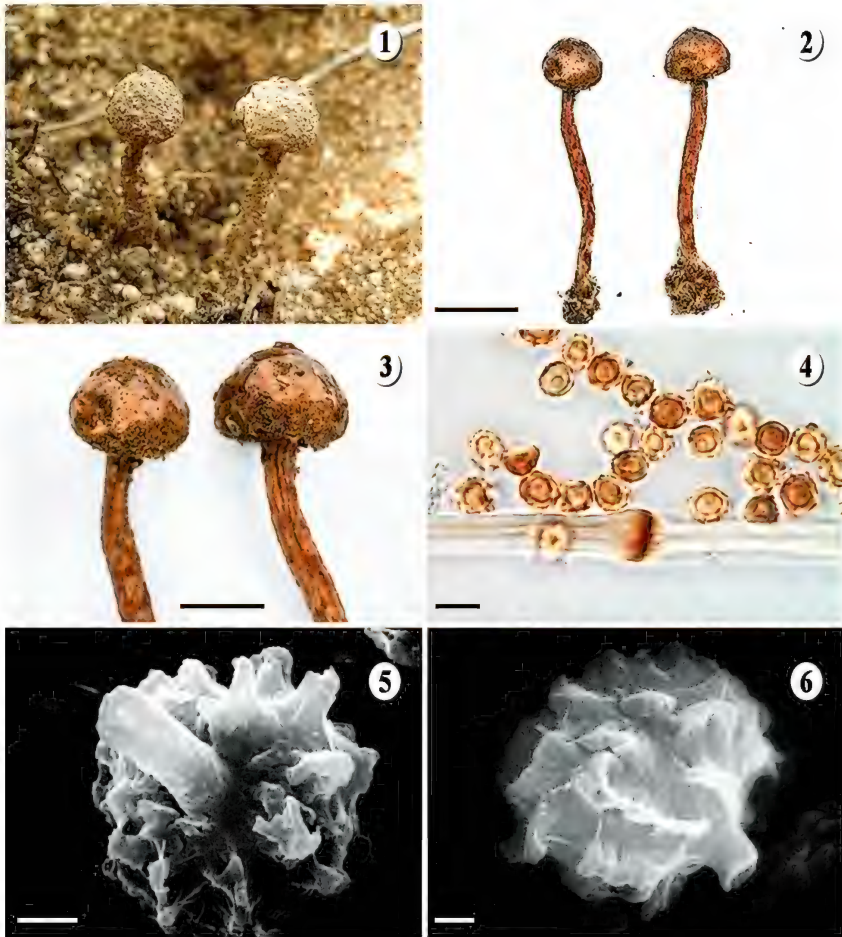
MYCOBANK MB 821805

Differs from *Tulostoma adhaerens* by its small to medium size spore-sacs, thinly membranous exoperidium persisting in patches in the pinkish endoperidium, and strongly echinulate basidiospores with the spines sometimes coalescing to form a subreticulum.

TYPE: Mexico, Sonora, municipality of Moctezuma, “La Madera,” 29°53'44"N 109°27'21"W, alt. 1214 m, sandy soil, in a subtropical scrub vegetation, 2 August 2010, leg. E. Hernández-Navarro, C. Piña, R. Maldonado & A. Gutiérrez (Holotype, UES 10528) (two complete basidiocarps); GenBank MF319226).

ETYMOLOGY: The name refers to the pinkish tone of the endoperidium.

SPORE-SAC 7.5–8.4 mm diam. × 5.7–5.9 mm high. EXOPERIDIUM thinly membranous, easily removable in big scales when dissected, dark because of sand and particles on the outside and whitish in the inside. Persistent in parts of the endoperidium like clay or scales, especially at the basal part of the spore-sac. ENDOPERIDIUM grayish pink (11B3–12B3), mottled with dark spots that



FIGS. 1–6. *Tulostoma rufescens* (holotype, UES 10528): 1–3. Basidiocarps; 4. Capillitium and spores (LM); 5, 6. Spore ornamentation (SEM). Scale bars: 2 = 10 mm; 3 = 5 mm; 4 = 5 μ m; 5, 6 = 1 μ m.

simulate warts due to the effect of the persistent exoperidium. MOUTH round to elliptic, 1×1.2 mm diam. and with a projection up to 0.4 mm in height. SOCKET conspicuous, deep, quite separate from the endoperidium, with a denticulated to lacerated membrane. GLEBA dark ferruginous to brown (8D4–8E8). STIPE partially buried in the sand, woody-fistulose, tortuous, $27\text{--}28 \times 1.7\text{--}1.8$ mm, light brown to reddish brown (6C4–7E8),

surface rugose-scaly, with a conspicuous basal bulb with hyphae strongly mixed with grains of sand and debris. SPORES globose to subglobose, reddish brown, echinulate, some spines slightly curved, some appearing subreticulate, 4.2–6.7 μm including ornamentation [mean = 5.1 μm , $Q_m = 0.99$ $n = 100$]; under SEM the ornamentation is formed by conspicuous conic structures, spines commonly fused in the apex, but some coalescing irregularly to a subreticulate pattern, with a pedicel variable in size. CAPILLITIUM subhyaline to slightly colored, 2–7 μm diam. [mean = 3.9 μm , $n = 100$], septa concolor with the spores, somewhat swollen 3–9.9 μm [mean = 5.5 μm $n = 100$].

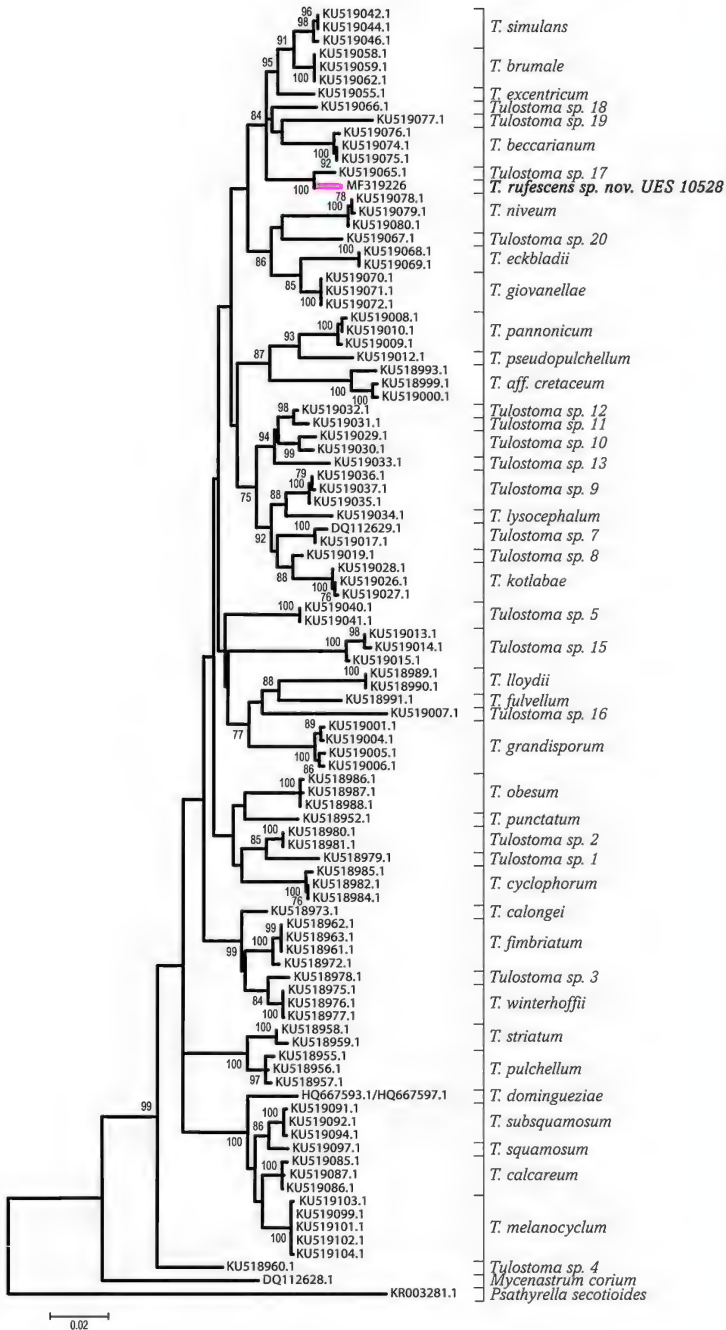
COMMENTS—Initial sequence homology tests by BLASTN of ITS region showed that the closest species is *Tulostoma* sp. 17 (Jeppson & al. 2017), an undescribed species from Hungary and Spain whose ITS barcode differs from our collection by 4% (data not shown). ML analyses of the complete ITS-5.8S-ITS and D1-D2 LSU regions placed our collection within the monophyletic genus *Tulostoma* (FIG. 7) and support its separation from similar morphospecies (e.g., *T. squamosum*).

When using distances in an NJ tree, the results are similar (FIG. 8). Some changes can be observed, especially concerning unnamed taxa and topology of lower branches. *Tulostoma* sp. 17 has not been described and its morphological traits are unknown; nonetheless, molecular analysis suggests that our collection is different from any sequenced *Tulostoma* species to date. It was also remarkable how the treatment of the gaps gave a better topology for NJ analysis than ML. This same result was observed with other algorithms (UPGMA, Minimum Evolution, Maximum Parsimony) when gaps were treated as pairwise deletions (data not shown).

Discussion

Tulostoma rufescens is distinguished by combination of the following characters: small to medium size spore-sac (<9 mm), pinkish endoperidium (a very uncommon color in the genus), tortuous stem with a conspicuous basal bulb, and the strongly echinulate spores that may coalesce to form a subreticulum in some spores.

FIG. 7. Molecular phylogenetic analysis of *Tulostoma* species based on the Maximum Likelihood method using the General Time Reversible model. The tree with the highest log likelihood (–8278.3120) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches, based on 1000 bootstrap replicates. The numbers after “*Tulostoma* sp.” refer to Jeppson & al. (2017). Our new sequence is set in bold.



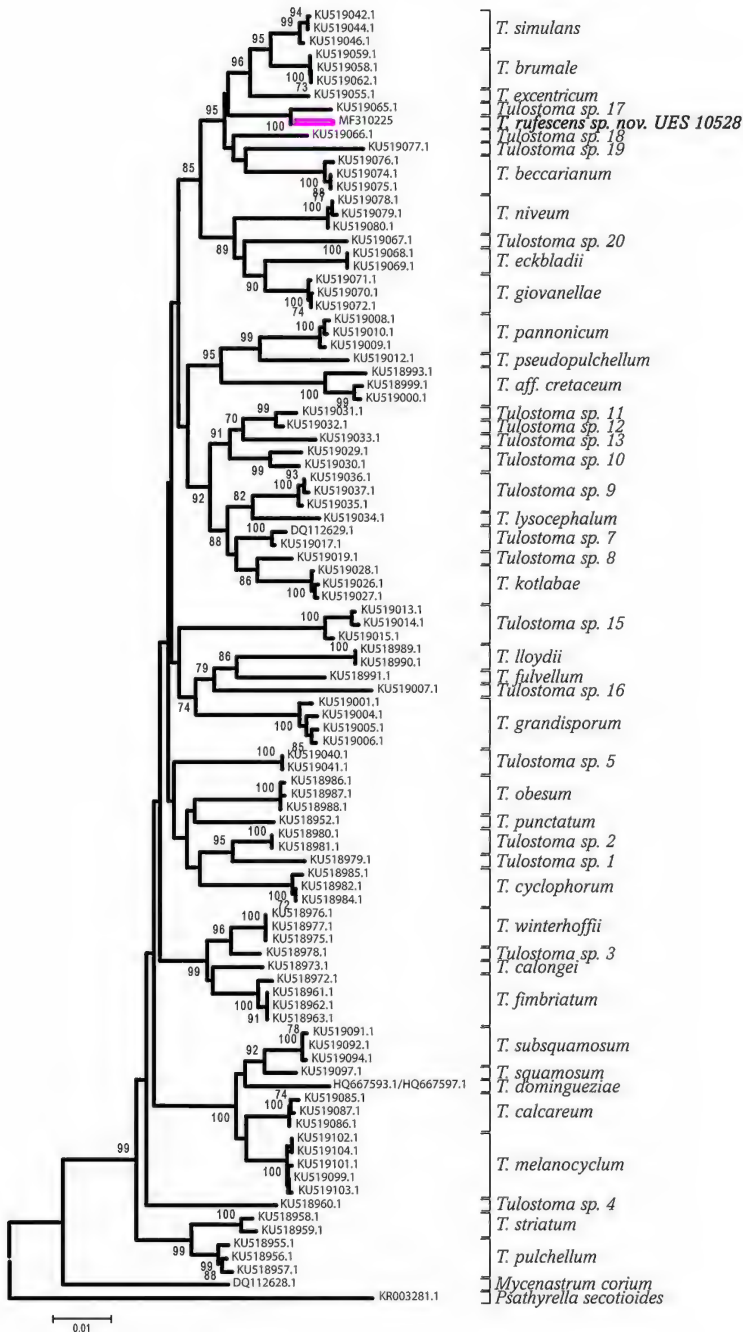
The closest morphospecies is possibly *T. adhaerens* Lloyd recorded from South Africa, Madagascar, Australia, Malaysia, and Japan, which differs by its indistinct exoperidium, more robust sporocarp, bigger spores (5.0–7.5 μm diam.), and epispore comprising independent spines, some of which are at the apex. Wright (1987) described the Australian holotype and isotype of *T. adhaerens* as medium sized (10–20 mm) with an indistinct exoperidium and subreticulate spores under LM but echinulate with independent spines in SEM. The Japanese material is close to 15 mm diam., with a spore ornamentation comprising several spines in coherent fascicles and each spine isolated at the base (Asai & Asai 2008). Wright (1987) designated *T. adhaerens* as the type of *Tulostoma* sect. *Hyphales* J.E. Wright based on its exoperidium with a thick layer of hyphae and a tubular or compressed cylindrical ostiole. Of the 37 species included in this section, 35 are accepted in Species Fungorum.

Tulostoma rufescens can also be confused with *T. squamosum* (J.F. Gmel.) Pers., which differs by its true verrucose exoperidium formed by irregular dark cells with a thick wall (sphaerocysts) and a squamous reddish brown stipe. A similar case would be *T. subsquamosum* Long & S. Ahmad, which also has spines but truly subreticulate spores in both LM and SEM, and an uncolored to ochraceous endoperidium. *Tulostoma rufescens* could also be confused with *T. beccarianum* Bres. and allied species such as *T. simulans* Lloyd, two taxa once synonymized based on morphology, but recently supported as independent by molecular data. The two species are separated morphologically from *T. rufescens* by their uncolored endoperidium and smaller spores with verrucae, not spines (Altés & Moreno 1993, Altés & al. 1996, Jeppson & al. 2017).

Tulostoma rufescens might be confused with *T. calcareum* Jeppson & al., which differs by its hyphal-verrucose exoperidium that is deciduous (but sometimes persisting as whitish scattered verrucae); its brownish-ochraceous endoperidium, initially rather dark colored but with age fading to greyish white; its greyish to brownish peristome; and its slightly smaller verrucose-echinate spores (4–6 μm , mean = 4.7–5.0 μm ; Jeppson & al. 2017).

There is a debate about gap management in phylogenetic analyses. It has been reported that treating indels as missing data in both Bayesian

FIG. 8. Molecular phylogenetic analysis of *Tulostoma* species based on the Neighbor-Joining method. The optimal tree is shown (with the sum of branch length = 0.89290951). The percentage of trees in which the associated taxa clustered together is shown next to the branches, based on 1000 bootstrap replicates. The numbers after "*Tulostoma* sp." refer to Jeppson & al. (2017). Our new sequence is set in bold.



and maximum likelihood phylogenetic estimations can be statistically inconsistent for determining a general and rather simple model of sequence evolution. Resulting priors on branch lengths and rate heterogeneity parameters may exacerbate the effects of ambiguous data, producing strongly misleading bipartition posterior probabilities, even while showing the true alignment (Nagy & al. 2012).

Some authors use a 3% threshold of sequence identity to determine conspecificity (Begerow & al. 2010); nevertheless, this value is not generally accepted for all fungi, since it has been shown to vary between groups, being too high for some taxa and too low for others (Nilsson & al. 2008). In both our analyses, bootstrap values were strong ($\geq 70\%$) in the higher branches, while some lower branches were not well supported ($\leq 70\%$). Low bootstrap values are related to evolutionary processes like incomplete lineage sorting and introgression of alleles across species boundaries (e.g. incomplete reproductive isolation) due to different selective processes (Morando & al. 2004).

Balasundaram & al. (2015) have suggested that the right DNA marker (or a particular combination of markers) and its intraspecific distances must be evaluated in order to reconstruct the accurate phylogeny of each group. The interspecific genetic distances must also be evaluated, as such distances are higher in geographically widely distributed species, stressing the importance of sampling more specimens from wider geographical ranges to determine intraspecific variation. In the absence of this information, the proposal of new names might not reflect cryptic diversification but rather taxonomic inflation, leading to a changed species concept rather than to new discoveries. This directly influences macroecology and conservation analyses, most of which are based on species lists (Isaac & al. 2004).

In addition, many researchers admit that the ITS region does not provide a precise species recognition for some fungal groups, such as for yeast, arbuscular mycorrhizas, and lichens. The two spacer regions (ITS1, ITS2) do not evolve independently because the variation of both regions is highly correlated, and 5.8S rRNA region is well conserved within all fungal species. However, the complete ITS meets the criterion for a good barcode marker: it is short (< 1000 bp), and there are several copies in the genome, making it easily amplified, even from degraded, environmental or old herbarium samples (Schoch & al. 2012). Other protein-coding genes might give a better species resolution but lack the many practical applications of ITS (Köljalg & al. 2013). The ITS region is repeatedly criticized for indel-induced alignment

problems and the lack of phylogenetic resolution above the species level, one reason why some authors chose to delete ambiguous characters from ITS and thereby losing potentially valuable information. However, it has been argued that ITS indels are slightly more conserved than nucleotide substitutions and when included in phylogenetic analyses improved the resolution and branch support, thus extending the resolving power of ITS (Nagy & al. 2012).

From a barcoding perspective and considering only the complete ITS region, currently fewer than 40 *Tulostoma* species have been properly sequenced, representing approximately 25% of known morphospecies. Despite the unusual characteristics of our collection, there are several examples of convergent evolution in fungi, where the molecular characterization of specimens has revealed cryptic species with shared morphological traits (Nguyen & al. 2013, 2016). *Tulostoma* is no exception, since common species such as *T. fimbriatum* are polyphyletic, supporting the idea that many of the morphological characters used for segregation of taxa are plesiomorphic or homoplastic due to convergent evolution or parallelism (Jeppson & al. 2017).

Nevertheless, further molecular analysis of the holotype of *T. adhaerens*, other *Tulostoma* holotypes, and *Tulostoma* specimens from Sonora and elsewhere are needed so as to verify the authenticity of names based on morphological species concepts, estimate the actual number of species, determine fully which morphological traits are taxonomically and phylogenetically informative, and understand the boundaries between closely-related species and genera.

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New records of six *Pyrenula* species from China

JIE-MENG FU ^{1#}, ZHONG-LIANG WANG ^{1#},
CHUN-XIAO WANG ¹, LU-LU ZHANG ^{2*}

¹Key Laboratory of Plant Stress Research, College of Life Sciences,
Shandong Normal University, Jinan, 250014, P. R. China

²Institute of Environment and Ecology, Shandong Normal University,
Jinan, 250014, P. R. China

* CORRESPONDENCE TO: jiemengfu11@outlook.com, wzl010118@outlook.com

ABSTRACT—*Pyrenula mastophora*, *P. micheneri*, and *P. thailandica* are reported for the first time from China; and *P. bahiana*, *P. complanata*, and *P. platystoma* are reported for the first time from mainland China. Descriptions, illustrations, and distributions are given for each species.

KEY WORDS—Asia, lichen-forming fungi, *Pyrenulaceae*, taxonomy

Introduction

The conserved genus *Pyrenula* Ach. (*Pyrenulaceae*, *Ascomycota*) was described by Acharius (1814). In a world key of *Pyrenula* species, Aptroot (2012) accepted 169 species out of the c. 745 named taxa in the genus. Since then, many new species of *Pyrenula* have been described and the genus now comprises c. 226 species (Ingle & al. 2018).

Pyrenula comprises crustose lichens typically growing on smooth, shaded bark (Aptroot 2012). It is characterized by a UV- or UV+ yellow thallus, perithecioid ascomata, an occasionally interspersed hamathecium, unbranched filaments, and distoseptate or (sub)muriform brown mature ascospores, and old spores with or without orange oil granules. Aptroot & al. (2013) noted that

[#] Jie-Meng Fu & Zhong-Liang Wang contributed equally to this paper.

the presence of orange reddish granules in over-mature spores is an important character that has made it much easier to recognize undescribed taxa.

In this paper we contribute to the knowledge of *Pyrenula* in China by providing fundamental data and reliable results in preparation for the Lichen Flora of China. We have identified three species new to China—*Pyrenula mastophora*, *P. micheneri*, and *P. thailandica*—and report for the first time three species from mainland China—*P. bahiana*, *P. complanata*, and *P. platystoma*.

Materials & methods

The specimens studied are preserved in Lichen Section of Botanical Herbarium, Shandong Normal University, Jinan, China (SDNU). The morphological and anatomical characters of the specimens were examined under a COIC XTL7045B2 stereo-microscope and an Olympus CX41 polarizing microscope. Unless otherwise indicated, sections were mounted in tap water (unless otherwise indicated); measurements were made in tap water or water enhanced with KOH. Lichen substances were identified using standardized thin layer chromatography (TLC) with system C (Orange & al. 2010). The lichens were photographed using Olympus SZX16 and BX61 microscopes and an Olympus DP72 camera.

Taxonomy

Pyrenula bahiana Malme, Ark. Bot. 22A(11): 26 (1929)

FIG. 1A–D

Thallus corticate, brownish to olive green, with pseudocyphellae, thallus UV–. Perithecia solitary, subglobose, immersed to erumpent from the substratum, often partly or mostly covered by thallus, 0.5–0.7 mm diam. Perithecial wall with crystals, up to 100 µm thick, often not fully carbonized. Ostiole pale, apical. Hamathecium not interspersed with oil droplets, filaments unbranched. Ascospores 8/ascus, grey brown, irregularly biseriate, 3-septate, fusiform with pointed ends, $27.5\text{--}30 \times 10\text{--}15$ µm, lumina angular to usually somewhat rounded, all lumina separated from the exospore by a thick endospore layer, old ascospores filled with red-orange oil. Substrate bark.

CHEMISTRY—No chemical substances detected by TLC.

SPECIMENS EXAMINED: CHINA. GUIZHOU, Jiangkou, Mt. Xiaoding, alt. 900 m, on bark, 4 Apr. 2016, Xiang-Xiang Zhao 20160694 (SDNU); alt. 850 m, on bark, 2 Apr. 2016, Xiang-Xiang Zhao 2016080 (SDNU).

DISTRIBUTION—Pantropical (Aptroot 2012). New to mainland China, but previously reported from Taiwan (Aptroot 2003).

COMMENTS—*Pyrenula bahiana* is characterized by the thallus with white pseudocyphellae, distoseptate ascospores with three septa, and over-mature

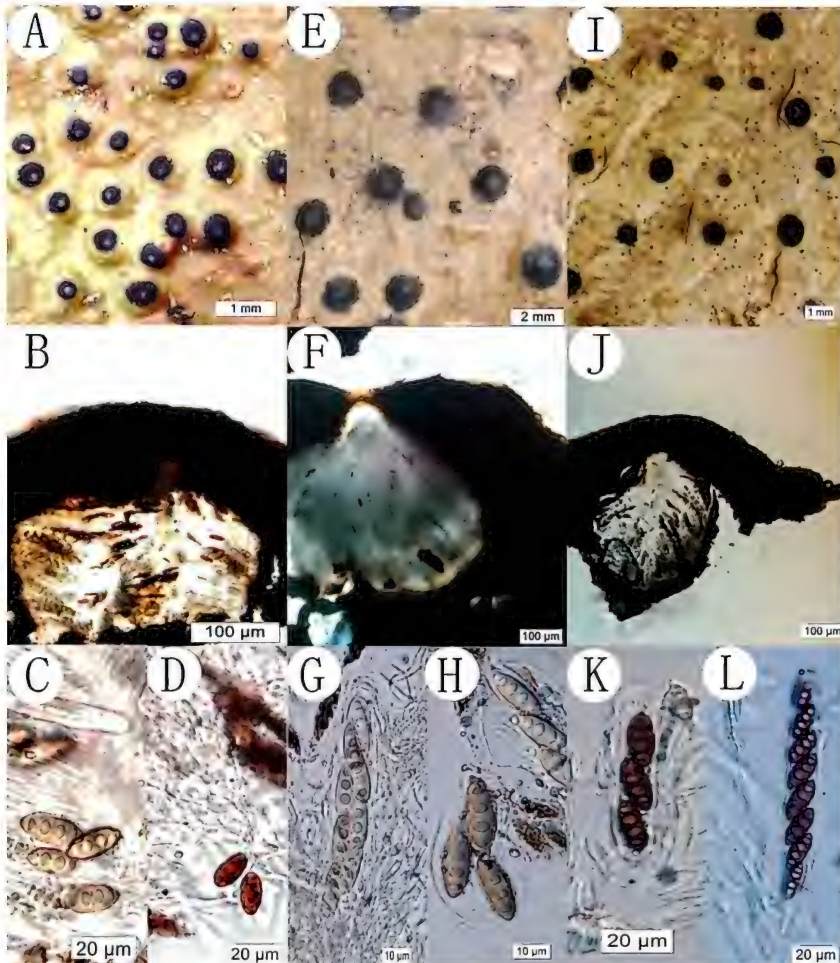


FIG. 1 *Pyrenula bahiana* (Zhao 20160694, SDNU). A: thallus with ascomata and pseudocyphellae; B: transverse section through ascoma; C: ascospores; D: over-mature spores with red-orange oil. *Pyrenula complanata* (Wang 20107042, SDNU). E: thallus with ascomata; F: transverse section through ascoma; G: ascus; H: ascospores. *Pyrenula mastophora* (Li 20110618, SDNU). I: thallus with ascomata; J: transverse section through ascoma; K: ascospores; L: ascus with 8 ascospores.

spores containing red-orange oil. It is closely related to *P. thailandica*, which has larger ascospores ($37.5\text{--}42.5 \times 15\text{--}17.5 \mu\text{m}$). Our specimens are similar to the *P. bahiana* protologue description, except that the holotype has larger ($29\text{--}38 \times 13\text{--}18 \mu\text{m}$) ascospores (Ingle & al. 2018).

Pyrenula complanata (Mont.) Trevis., Spighe e Paglie: 17 (1853) FIG. 1E–H

Thallus corticate, pale brownish, without pseudocyphellae, thallus UV–. Perithecia mostly simple, only aggregated as by chance when crowded, erumpent from the substratum, perithecia subglobose and spreading laterally, 0.9–1.4 mm diam. Perithecial wall <225 µm. Ostiole black, apical. Hamathecium not interspersed with oil droplets, filaments unbranched. Ascospores 8/ascus, grey brown, 3-septate, ellipsoid with rounded ends, 30–32.5 × 10–12.5 µm, end lumina separated from the exospore by a thick endospore layer, lumina angular. Substrate bark.

CHEMISTRY—No chemical substances detected by TLC.

SPECIMENS EXAMINED: CHINA. GUANGXI, Laibinjinxiu, Mt. Shengtang, alt. 1400 m, on bark, 8 Nov. 2010, Hai-Ying Wang 20107042 (SDNU). FUJIAN, Longyan, Mt. Huanglianyu, alt. 600 m, on bark, 2 Apr. 2010, Xing-Ran Kou 20105891 (SDNU). YUNNAN, Jingdong, Mt. Ailao, Dujuanhu, alt. 2500 m, on bark, 5 Aug. 2017, Rong Tang & al. 20170335 (SDNU).

DISTRIBUTION—Pantropical (Aptroot 2012). New to mainland China, but previously reported from Taiwan (Aptroot 2003).

COMMENTS—*Pyrenula complanata* is characterized by the thallus lacking pseudocyphellae and distoseptate ascospores with three septa. It is closely related to *P. media*, which has shorter ascospores (24–29 µm) and a thicker thallus (Aptroot 2012). Our specimens are closely similar to the *P. complanata* protologue description (Montagne 1843).

Pyrenula mastophora (Nyl.) Müll. Arg., Flora 66: 426 (1883) FIG. 1I–L

Thallus corticate, pale brownish, without pseudocyphellae, thallus UV–. Perithecia simple, solitary, erumpent from the substratum, 0.7–0.9 mm diam. Perithecial wall up to 200 µm. Ostiole black, apical. Hamathecium not interspersed with oil droplets, filaments unbranched. Ascospores 8/ascus, grey brown, 3-septate, ellipsoid with rounded ends, 25–27.5 × 10–12.5 µm, Central lumina much wider than long, end lumina separated from the exospore by a thick endospore layer. Substrate bark.

CHEMISTRY—No chemical substances detected by TLC.

SPECIMENS EXAMINED: CHINA. GUANGXI, Baise, Tianlin, Mt. Cenwanglao, alt. 2000 m, on bark, 23 Mar. 2011, Lin Li 20110618 (SDNU). GUIZHOU, Kaili, Mt. Leigong, alt. 2130 m, on bark, 1 Nov. 2009, Zun-Tian Zhao (SDNU).

DISTRIBUTION—Pantropical (Aptroot 2012). New to China.

COMMENTS—*Pyrenula mastophora* is characterized by the thallus without pseudocyphellae, distoseptate ascospores with three septa and wide central

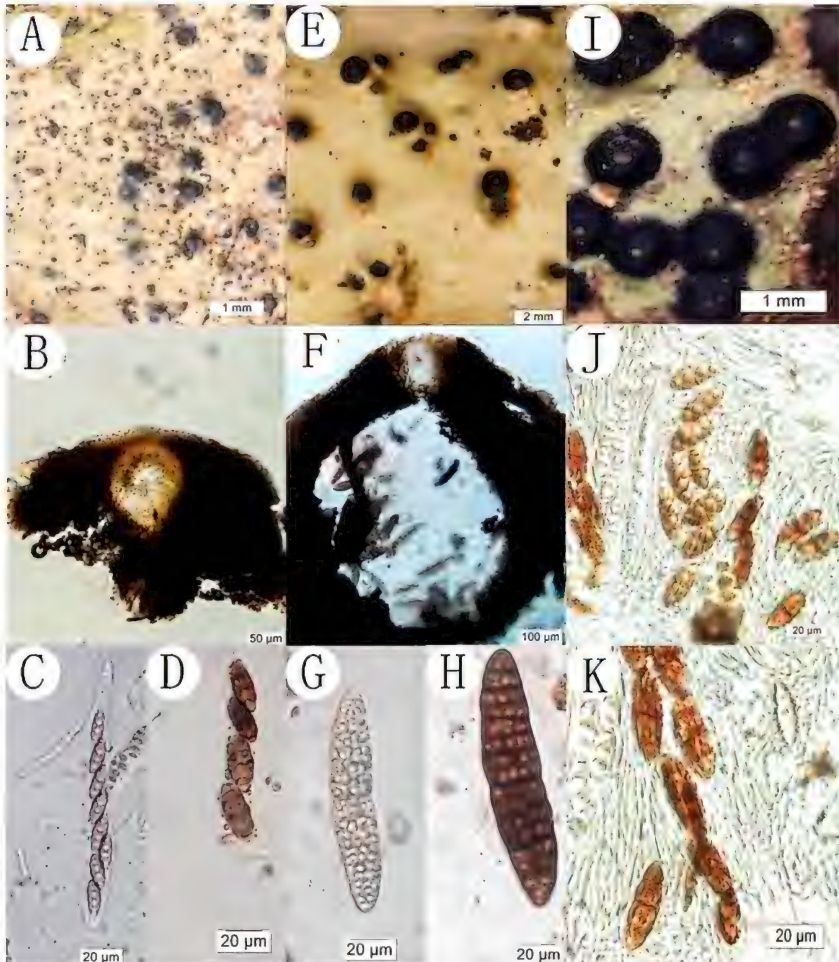


FIG. 2 *Pyrenula micheneri* (Li 20106097, SDNU). A: thallus with ascomata; B: transverse section through ascoma; C: ascus; D: ascospores. *Pyrenula platystoma* (Tang & al. 20171074, SDNU). E: thallus with ascomata; F: transverse section through ascoma; G: Immature ascospore; H: mature ascospore. *Pyrenula thailandica* (Wang 20102341, SDNU). I: thallus with ascomata and pseudocyphellae; J: ascospores; K: over-mature spores with red-orange oil.

lumina, and non-conical ascomata. It is closely related to *P. quassiicola*, which has more or less rounded central lumina and often possesses pseudocyphellae. Our specimens match the *P. mastophora* protologue description, except that the holotype has conical ascomata (Aptroot 2012).

Pyrenula micheneri R.C. Harris,

Mem. New York Bot. Gard. 49: 96 (1989)

FIG. 2A–D

Thallus corticate, pale brownish to olive green, without pseudocyphellae, thallus UV–. Perithecia simple, only aggregated as by chance when crowded, immersed to erumpent from the substratum, often partly or mostly covered by thallus, perithecia subglobose, 0.4–0.6 mm diam. Ostiole pale, apical, with numerous crystals around ostioles. Perithecial wall with crystals, <150 µm. Hamathecium not interspersed with oil droplets, filaments unbranched. Ascospores 8/ascus, grey brown, 3-septate, ellipsoid with rounded ends, with angular diamond-shaped lumina, 22.5–27.5 × 7.5–10 µm, end lumina separated from the exospore by a thick endospore layer. Substrate bark.

CHEMISTRY—No chemical substances detected by TLC.

SPECIMEN EXAMINED: CHINA. JIANGXI, Jian, Qianmo, Nanfengmian, alt. 1300 m, on bark, 2 Nov. 2010, Ming Li 20106097 (SDNU).

DISTRIBUTION—Temperate America (Aptroot 2012). New to China.

COMMENTS—*Pyrenula micheneri* is characterized by a thallus without pseudocyphellae, an apical ostiole, numerous crystals surrounding the ostiole, and distoseptate ascospores with three septa and angular diamond-shaped lumina. It is closely related to *P. pyrgillospora*, which has rounded or quadrangular lumina (Aptroot 2012). Our specimen closely matches the *P. micheneri* protologue description (Harris 1989).

Pyrenula platystoma (Müll. Arg.) Aptroot, Lichenologist 44: 36 (2012) FIG. 2E–H

Thallus corticate, yellowish brown, without pseudocyphellae, thallus UV–. Perithecia simple, only aggregated as by chance when crowded, erumpent from the substratum, often partly covered by thallus, 0.7–1.0 mm diam. Perithecial wall <250 µm. Ostiole pale, apical. Hamathecium not interspersed with oil droplets, filaments unbranched. Ascospores 2/ascus, grey brown, muriform with 8–10 rows, fusiform with rounded ends, 145–150 × 28.5–30 µm. Substrate bark.

CHEMISTRY—No chemical substances detected by TLC.

SPECIMEN EXAMINED: CHINA. YUNNAN, Midu, Mt. Taijiding, alt. 2650 m, on bark, 8 Aug. 2017, Rong Tang & al. 20171074 (SDNU).

DISTRIBUTION—Pantropical (Aptroot 2012). New to mainland China, but previously reported from Taiwan as *Anthracothecium oculatum* Müll. Arg. (Zahlbruckner 1940, Lamb 1963, Wang & Lai 1973, Kashiwadani & Kurokawa 1981, Wei 1991), which is a synonym of *P. platystoma* (Aptroot 2012).

COMMENTS—*Pyrenula platystoma* is characterized by the thallus without pseudocyphellae and large muriform ascospores. It is closely related to *P. neosandwicensis*, which has pseudocyphellae (Aptroot 2012). Our specimen matches the *P. platystoma* protologue description, except that the holotype has mostly shorter (80–140(–155) μm long) ascospores (Aptroot 2012).

Pyrenula thailandica Aptroot, Lichenologist 44: 617 (2012)

FIG. 2I–K

Thallus corticate, brownish green, with pseudocyphellae, thallus UV–. Perithecia solitary, subglobose, erumpent from the substratum, 0.7–0.8 mm diam. Ostiole pale, apical. Hamathecium not interspersed with oil droplets, filaments unbranched. Ascospores 8/ascus, grey brown, 3-septate, fusiform with pointed ends, $37.5\text{--}42.5 \times 15\text{--}17.5 \mu\text{m}$, lumina angular to usually somewhat rounded, end lumina separated from the exospore by a thick endospore layer, over-mature ascospores filled with red-orange oil. Substrate bark.

CHEMISTRY—No chemical substances detected by TLC.

SPECIMEN EXAMINED: CHINA. GUIZHOU, Libo, Maolan protection zone, alt. 500 m, on bark, 2 Nov. 2009, Hai-Ying Wang 20102341 (SDNU).

DISTRIBUTION—Eastern palaeotropical (Aptroot & al. 2012). New to China.

COMMENTS—*Pyrenula thailandica* is characterized by the thallus with white pseudocyphellae, distoseptate ascospores with three septa, and old spores with red-orange oil. It is closely related to *P. bahiana*, which has smaller ascospores ($25\text{--}27.5 \times 10\text{--}15 \mu\text{m}$). Our specimen matches the *P. thailandica* protologue description, except that the holotype has $(30\text{--})35\text{--}51 \times (10\text{--})14\text{--}20 \mu\text{m}$ ascospores (Aptroot & al. 2012).

Acknowledgements

The authors thank Dr. Aptroot A (ABL Herbarium, The Netherlands) and Dr. Shou-Yu Guo (State Key Laboratory of Mycology, Institute of Microbiology, Chinese Academy of Sciences, Beijing) for specimen identification guidance and presubmission reviews. We also thank Dr. Aptroot for providing great help during the study. This study was supported by the National Natural Science Foundation of China (31400015, 31570017) and the Undergraduate Scientific Research Foundation of Shandong Normal University (2017BKSKY61).

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***Cantharocybe virosa*, first record of the genus in Thailand**

JATURONG KUMLA, NAKARIN SUWANNARACH, SAISAMORN LUMYONG*

*Department of Biology, Faculty of Science, Chiang Mai University,
Chiang Mai, 50200, Thailand*

* CORRESPONDENCE TO: saisamorn.l@cmu.ac.th

ABSTRACT—Mushroom specimens collected in Thailand were identified as *Cantharocybe virosa* based on morphology and similarities of LSU and ITS genes. A full description, color images, and line drawing are provided. This is the first record of the genus *Cantharocybe* from Thailand.

KEY WORDS—*Agaricales*, gill fungus, *Hygrophoraceae*, taxonomy

Introduction

Cantharocybe (*Hygrophoraceae*, *Agaricales*) was described by Bigelow & Smith (1973) with *C. gruberi* (A.H. Sm.) H.E. Bigelow & A.H. Sm. as the type species. This genus has a worldwide distribution in temperate and tropical regions. Three species of *Cantharocybe* have been described: *C. brunneo-velutina*, *C. gruberi*, and *C. virosa*; they are saprotrophic and usually grow on soils (Bigelow & Smith 1973, Manimohan & al. 2010, Ovrebo & al. 2011, Kumar & Manimohan 2013). Although no species of *Cantharocybe* has been reported from Thailand (Chandrasrikul & al. 2011), during a taxonomic survey of macrofungi collected in northern Thailand, we found specimens that corresponded to the description of *C. virosa*, previously reported from Bangladesh and India (Manimohan & al. 2010, Kumar & Manimohan 2013, Hosen & al. 2016, Acharya & al. 2017). Here we describe and illustrate the morphological characteristics of the Thai material and cite supporting evidence from LSU and ITS gene sequence matches in GenBank.

Materials & methods

Morphology studies

Basidiocarps were collected from the campus of Chiang Mai University, Chiang Mai Province, Thailand, and wrapped in aluminum foil or kept in plastic collection boxes until transported back to the laboratory; notes on macromorphological features and photographs were taken within 24 h of collection. Color names and codes follow Kornerup & Wanscher (1978). The micromorphological data were derived from dried specimens mounted in 95% ethanol followed by distilled water, 3% KOH, or Melzer's reagent. Dimensions of anatomical features were from at least 50 measurements of each structure. The specimens were dried at 40–45 °C on an electric food dryer and deposited in the herbarium of Research Laboratory for Excellence in Sustainable Development of Biological Resources, Faculty of Science, Chiang Mai University, Thailand (SDBR-CMU).

Molecular studies

Genomic DNA was extracted from a fresh specimen using a Favorgen DNA Extraction Mini Kit following the manufacturer's instructions. The large subunit (LSU) region of ribosomal DNA (rDNA) was amplified by polymerase chain reaction (PCR) with LROR/LR5 primers under the following thermal conditions: 94 °C for 2 min; 35 cycles of 95 °C for 30 s, 52 °C for 30 s, 72 °C for 1 min; and 72 °C for 10 min. The internal transcribed spacer (ITS) rDNA region was amplified using ITS4/ITS5 primers under the previously listed thermal conditions. Negative controls lacking fungal DNA were run to check for reagent contamination. PCR products were checked on 1% agarose gels stained with ethidium bromide under UV light and purified using a Macherey-Nagel NucleoSpin® Gel and PCR Clean-up Kit following the manufacturer's protocol. The purified PCR products were directly sequenced. Sanger sequencing was performed by 1ST Base Company in Kembangan, Malaysia using the same PCR primers mentioned above. Sequences were used to query GenBank database via BLAST (<http://blast.ddbj.nig.ac.jp/top-e.html>).

Taxonomy

Cantharocybe virosa (Manim. & K.B. Vrinda) T.K.A. Kumar,
Mycotaxon 124: 235 (2013).

FIG. 1

Pileus 30–85 mm diam., convex, becoming broadly convex; surface light brown (6D5) to grayish brown (7E3) or dark grey (7F8), slightly darker at the centre; margin inrolled when young, becoming incurved and finally becoming straight. Lamellae adnate to decurrent, <1.5 mm wide, white (4A1) to yellowish white (1A2). Stipe 30–60 × 5–20 mm, central or at times slightly excentric, solid, cylindrical or tapering towards the base, concolorous with the pileus, with cottony mycelium at the base. Odor strong and unpleasant. Spore print white.

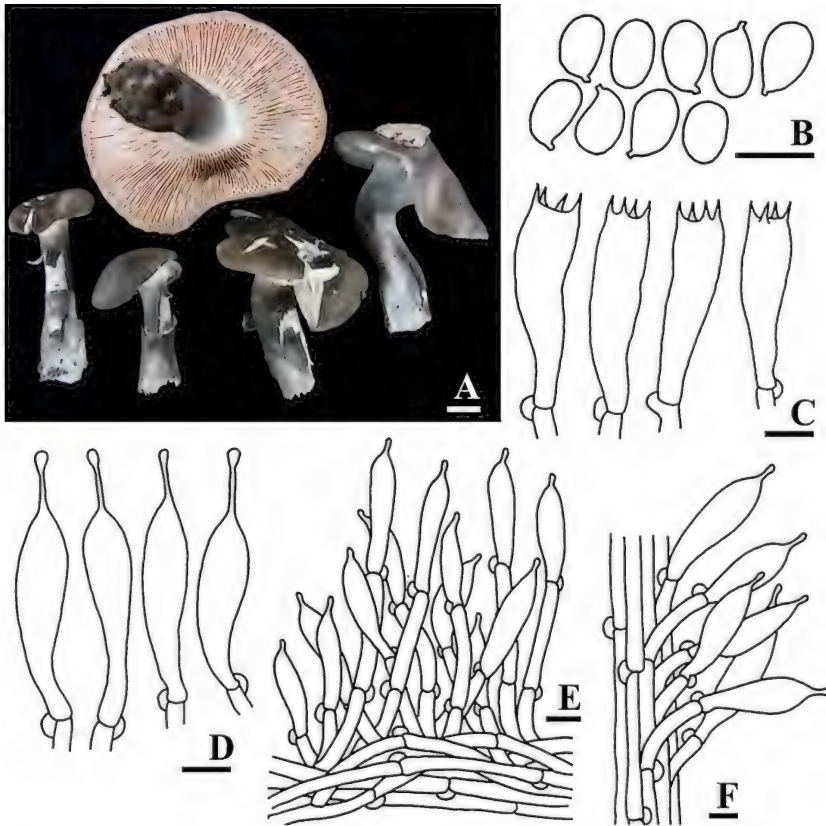


FIG. 1. *Cantharocybe virosa* (SDBR-CMU-M0145). A: basidiocarps; B: basidiospores; C: basidia; D: cheilocystidia; E: trichoderm pileipellis; F: surface of stipe in longitudinal section showing caulocystidia. Scale bars: A = 10 mm, B–F = 10 µm.

Basidiospores $6\text{--}11 \times 5\text{--}7 \mu\text{m}$ ($n = 50$), $Q = 1.2\text{--}1.7$, subglobose to ellipsoid, thin-walled, smooth, with refractive guttules, inamyloid. Basidia $22\text{--}55 \times 6\text{--}12 \mu\text{m}$, clavate, 4-spored, with basal clamp connections, sterigmata $<5 \mu\text{m}$. Pleurocystidia absent. Cheilocystidia abundant, $20\text{--}65 \times 5\text{--}9 \mu\text{m}$, lecythiform to lageniform, sometimes with a mucronate apex, base portion usually clavate, the upper portion extending into an elongated neck $<35 \mu\text{m}$ long with or without a rounded capitulum, thin-walled, hyaline to pale yellow. Lamellar trama parallel to sub-regular, composed of branching hyphae $3\text{--}15 \mu\text{m}$ diam., septate, thin-walled, hyaline to pale yellow. Pileipellis a trichoderm, interwoven,

composed of hyphae 4–10 µm diam., septate, thin- to slightly thick-walled, often pale brown vacuolar to plasmatic pigment; terminal cells 20–60 × 3–10 µm, cylindrical to narrowly clavate; pileocystidia with or without extending neck, with one or two short rounded capitula, elongated neck <15 µm long. Stipitipellis composed of branching hyphae 2–10 µm diam., septate, thin- to slightly thick-walled, outer surface more or less covered with cylindrical to narrow clavate cells 30–100 × 6–10 µm, with or without a rounded capitulum head, pale brown vacuolar to plasmatic pigments. Clamp connections present in all tissue types.

SPECIMENS EXAMINED—THAILAND, CHIANG MAI PROVINCE, Muang District, Chiang Mai University, 18°48'00"N 98°57'21"E, elevation 334 m, on soil, 4 September 2015, Kumla J. & Suwannarach N. (SDBR-CMU-M0145; GenBank MG694689, MG694691); 4 October 2017 Suwannarach N. (SDBR-CMU-NK0280, GenBank MG694690, MG694692).

Molecular analysis

The LSU and ITS sequences of specimens SDBR-CMU-M0145 and SDBR-CMU-NK0280 were deposited in the GenBank database. Our LSU sequences (MG694689, MG694690) were 100% similar to the *C. virosa* TENN 63483 ex-paratype sequence JX101471; and our ITS sequences (MG694691, MG694692) were 99% similar to the *C. virosa* TENN 63483 ex-paratype sequence KX452405.

Discussion

The clitocybeoid light brown to dark grey basidiocarps, abundant lecythiform or mucronate cheilocystidia, and the subglobose to ellipsoid smooth inamyloid basidiospores support placement of our collections in *Cantharocybe* (Bigelow & Smith 1973, Lodge & al. 2014). The two specimens collected in northern Thailand were initially identified as *C. virosa* after consulting the descriptions by Kumar & Manimohan (2013) and Acharya & al. (2017) and keys by Kumar & Manimohan (2013) and Hosen & al. (2016). The pale yellow to lemon yellow pileus and narrowly elliptical to oblong (11–17.5 × 4.5–7.5 µm) basidiospores of *C. gruberi* (Bigelow & Smith 1973) clearly distinguish that species from *C. virosa*. *Cantharocybe brunneovelutina* Lodge & al. differs from *C. virosa* by its velutinous basidiocarps, cheilocystidia with multiple prong-like appendages at the apex resembling a basidia-like structure, and shorter (9–9.5 × 5.5–6 µm) basidiospores (Ovrebo & al. 2011). The LSU and ITS molecular analyses support the morphological differences (Hosen & al. 2016).

Geographical distributions also support three species: *C. virosa* has been reported in tropical southern Asia (Bangladesh; India) and is a known toxic species (Manimohan & al. 2010, Kumar & Manimohan 2013, Hosen & al. 2016, Acharya & al. 2017). *Cantharocybe gruberi* is a widely distributed northern temperate species recorded from western North America (British Columbia; western U.S.A.) and Europe (Spain) (Bigelow & Smith 1973, Hosen & al. 2016). *Cantharocybe brunneovelutina* is known only from its type locality in tropical Central America (Belize; Ovrebø & al. 2011).

The ITS and LSU sequence matches in GenBank confirmed that the two Thai specimens belong to *C. virosa*. The combination of morphological and molecular characteristics supports the identification of *C. virosa*, a new record in Thailand.

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***Arthonia*, *Byssoloma*, *Calenia*, *Chroodiscus*, *Coenogonium*, *Eremothecella*, and *Semigyallecta* spp. new to China**

WEI-CHENG WANG^{1, 2} & JIANG-CHUN WEI^{1, 2 *}

¹ State Key Laboratory of Mycology, Institute of Microbiology,
Chinese Academy of Sciences, Beijing 100101, China

² University of Chinese Academy of Sciences, Beijing 100049, China

* CORRESPONDENCE TO: weijc2004@126.com weicheng88lichen@outlook.com

ABSTRACT—Ten foliicolous lichens are newly reported for China—*Arthonia accolens*, *A. cyanea*, *A. palmulacea*, *Byssoloma annuum*, *B. subleucoblepharum*, *Calenia lueckingii*, *Chroodiscus argillaceus*, *Coenogonium minimum*, *Eremothecella macrosperma*, and *Semigyallecta paradoxa*. Except for *C. minimum* collected from Guangxi Province, these species were collected from a rain forest in Hainan Province. Descriptions and photos of the ten species and comments are provided.

KEY WORDS—Asia, *Gomphillaceae*, *Graphidaceae*, *Gyallectaceae*, *Pilocarpaceae*

Introduction

Foliicolous lichens are mainly crustose lichens inhabiting the surface of living leaves of plants. More than 800 species are presently known in the world, which are found primarily in tropical and subtropical regions (Lücking 2008). Foliicolous lichens are common in south China, mainly in the provinces of Hainan, Yunnan, Guangxi, and Guangdong, as well as in Taiwan. Although foliicolous lichen diversity is rather rich in these regions, it lacks detailed study; in fact, only a few articles have been published on the foliicolous lichens of China (Sérusiaux 1976, Wei & Jiang 1991, Aptroot & al. 2003, Jiang & al. 2016). Here we present the results of a study of specimens collected from Hainan and Guangxi Provinces by various collectors. All the specimens are stored at HMAS-L. *Arthonia*, *Byssoloma*, and *Coenogonium* were found to be the most

common genera and deserve particular attention. Here, we present ten new records based on our research on foliicolous lichens of China.

Materials & methods

Specimens were collected from Hainan Province by Jiang Yu-Mei & Guo Shou-Yu in 1993; Huang Man-Rong in 2000; Wei Jiang-Chun in 2000, 2006, 2007; Zhang Tao in 2007; Wei Xin-Li & Jiang Shu-Hua in 2014; and Wang Wei-Cheng in 2017 and from Guangxi Province by Jiang Shu-Hua in 2014. They are preserved in the herbarium of Institute of Microbiology, Chinese Academy of Sciences, Beijing, China (HMAS-L). Morphology and anatomy were examined under a Motic stereomicroscope and a Zeiss Axioskop 2 plus microscope. Morphological photographs were taken with a Leica M125 dissecting microscope equipped with a Leica DFC450 camera, and anatomical photographs were taken with a Zeiss Imager A2 compound microscope equipped with a Zeiss AxioCam MRc5 Camera.

Taxonomy

Arthoniaceae Rchb.

Arthonia accolens Stirt.,

Proc. Roy. Philos. Soc. Glasgow 11: 105 (1879)

FIG. 1A, B

THALLUS dispersed into round patches, but usually connected together in the central part, yellow-brown, smooth, margin distinct. ASCOMATA not raised above thallus level, dark red-brown, round, 0.3–0.7 mm diam., about 50 μm high; epihymenium brown; hymenium and hypothecium pale brown. ASCI 8-spored, short clavate; ascospores clavate, 2-septate, distal cell enlarged, not curved, with slight constrictions at septa, colorless, $12.5\text{--}15 \times 3\text{--}5 \mu\text{m}$ ($n = 120$).

CHEMISTRY—not tested.

SPECIMENS EXAMINED: CHINA. HAINAN, Ledong Co., Jianfeng Ridge, Mingfeng Valley, $18^{\circ}44'36''\text{N } 108^{\circ}50'39''\text{E}$, 960 m a.s.l., on leaves, 6 Sep. 2017, Wei C. Wang 139434, 139435 (HMAS-L); Baoting Co., Qixian Ridge National Forest Park, $18^{\circ}42'21''\text{N } 109^{\circ}42'14''\text{E}$, 350 m a.s.l., on leaves, 7 Sep. 2017, Wei C. Wang 139424, 139426 (HMAS-L); 500 m a.s.l., on leaves, 7 Sep. 2017, Wei C. Wang 139425 (HMAS-L); Wuzhishan City, Wuzhishan National Forest Park, $18^{\circ}54'18''\text{N } 109^{\circ}41'04''\text{E}$, 800 m a.s.l., on leaves, 8 Sep. 2017, Wei C. Wang 139432 (HMAS-L).

DISTRIBUTION—Kenya (Lücking & Kalb 2002), Vietnam (Nguyen & al. 2011), and China (Hainan).

COMMENTS—*Arthonia accolens* is recognized by its shiny yellow-brown thallus, distinctly marginate red-brown ascomata, and 2-septate ascospores. The same ascospore type is found in *A. lividula*, which is distinguished by its light brown,

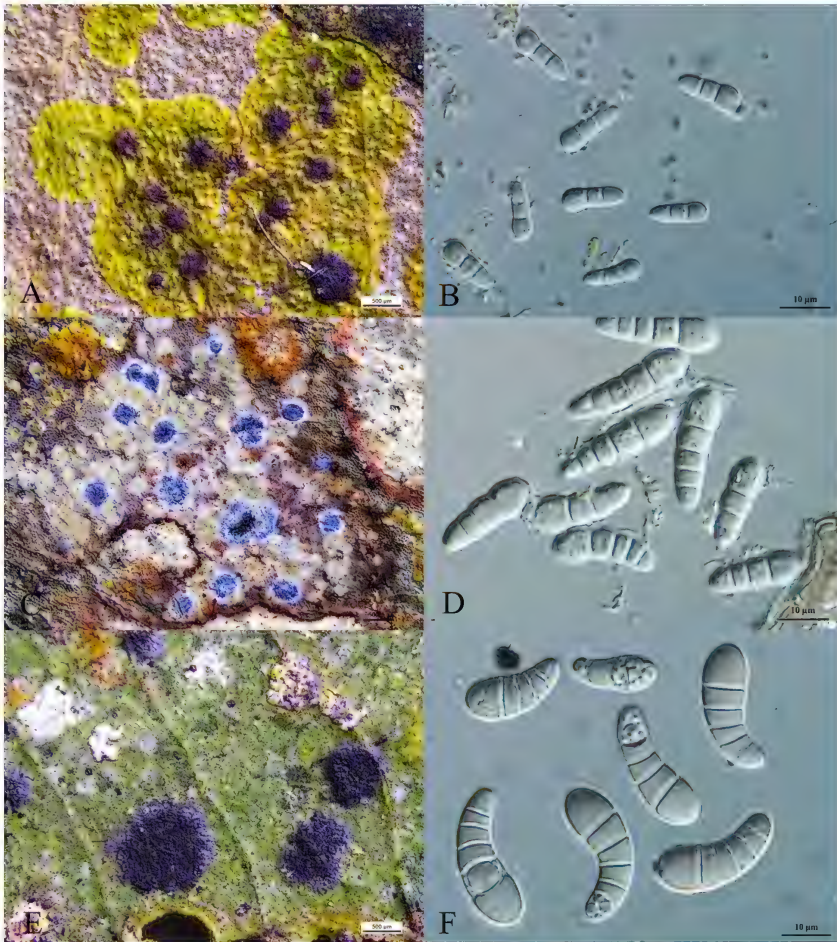


FIG. 1. Habitus and ascospores. A, B: *Arthonia accolens* (HMAS-L Wang 139432); C, D: *Arthonia cyanea* (HMAS-L Jiang & Guo 138619); E, F: *Arthonia palmulacea* (HMAS-L Wang 139415). Scale bars: A, C, E = 500 µm; B, D, F = 10 µm.

smaller ascomata (<0.5 mm diam.) with diffuse margins. *Arthonia accolens* is common in Hainan.

Arthonia cyanea Müll. Arg., Flora 64: 233 (1881)

FIG. 1C, D

THALLUS dispersed into small round patches, green-grey, thin, smooth. ASCOMATA not raised above thallus level, bluish grey, with white pruina, round or slightly irregular in outline, 0.4–1.0 mm diam., about 50 µm high;

epihyemenium composed of pale yellow granules, with crystals; hyemenium and hypothecium colorless. ASCI 8-spored, short clavate; ascospores clavate, 3–4-septate, distal cell enlarged, with constrictions at septa, colorless, $17.5\text{--}25 \times 5\text{--}7 \mu\text{m}$ ($n = 120$).

CHEMISTRY—not tested.

SPECIMENS EXAMINED: CHINA. HAINAN, Ledong Co., Jianfeng Ridge, $18^{\circ}44'N$ $109^{\circ}10'E$, 900 m a.s.l., on leaves, 1–3 Apr. 1993, Yu M. Jiang & Shou Y. Guo 138607, 138619, 138620, 138609, 138610 (HMAS-L); on leaves, 2 Oct. 2008, B. Gao 126474 (HMAS-L); Changjiang Co., Bawang Ridge, $19^{\circ}15'N$ $109^{\circ}02'E$, 670 m a.s.l., on leaves, 20 May 2007, T. Zhang 126740 (HMAS-L); $19^{\circ}16'N$ $109^{\circ}03'E$, on leaves, 22 Apr. 2000, Jiang C. Wei 112772 (HMAS-L).

DISTRIBUTION—Rwanda (Bock & al. 2007), Tanzania (Farkas 2015), Kenya (Lücking & Kalb 2002), Congo (Van den Broeck & al. 2014), Madagascar (Aptroot 2016), New Zealand (Galloway 2007), Australia, India, Indonesia, Philippines (Santesson & Tibell 1988), Indonesia (Santesson & Tibell 1988), Thailand (Papong & al. 2007), Malaysia (Sipman 1993), and China (Hainan).

COMMENTS—*Arthonia cyanea* is a widely distributed species, characterized by its patched thallus, bluish grey pruinose ascomata, and 2–4-septate clavate ascospores. Our Hainan specimens differ slightly from previous descriptions of *A. cyanea*, which do not report any 4-septate ascospores; but they are closer to typical *A. cyanea* than to its two segregate infraspecific taxa.

Arthonia palmulacea (Müll. Arg.) R. Sant.,

Symb. Bot. Upsal. 12(1): 87 (1952)

FIG. 1E, F

THALLUS dispersed into small round patches, green-grey, thin, smooth. ASCOMATA not raised above thallus level, black, without pruina, round, 0.3–1.0 mm diam., about $40 \mu\text{m}$ high; hyemenium pale brown, hypothecium brown. ASCI 8-spored, globose; ascospores clavate, 3–5-septate, distal cell enlarged, usually curved, without distinct constrictions at septa, colorless, $16\text{--}28 \times 8 \mu\text{m}$ ($n = 80$).

CHEMISTRY—not tested.

SPECIMEN EXAMINED: CHINA. HAINAN, Wuzhishan City, Mt. Wuzhishan, tropical rain forest scenic spot, $18^{\circ}51'59"N$ $109^{\circ}40'30"E$, 600 m a.s.l., on leaves, 9 Sep. 2017, Wei C. Wang 139415 (HMAS-L).

DISTRIBUTION—Congo (Van den Broeck & al. 2014) and China (Hainan).

COMMENTS—*Arthonia palmulacea* is easily recognized by its black ascomata and 3–5-septate curved ascospores lacking septal constrictions.



FIG. 2. Habitats and ascospores. A, B: *Eremothecella macrosperma* (HMAS-L Wang 139408); C, D: *Byssoloma annuum* (HMAS-L Wang 139408); E, F: *Byssoloma subleucoblepharum* (HMAS Gao 126449). Scale bars: A, C = 500 µm; B, D, F = 10 µm; E = 200 µm.

Eremothecella macrosperma (Zahlbr.) Sérus,
Syst. Ascom. 11: 43 (1992)

FIG. 2A, B

THALLUS smooth, greyish green. Photobiont cells orange, bead-like, about 12.5 µm diam. ASCOMATA black, <1 mm diam. and 120 µm high. Epithymenium, hymenium and hypothecium are all colorless or (at most) pale brown. Asci 8-spored; ascospores clavate, large, 7–11-septate, usually curved, distal cell enlarged, with constrictions at septa, colorless, 35–60 × 10 µm (n = 60).

CHEMISTRY—not tested.

SPECIMEN EXAMINED: CHINA. HAINAN, Wuzhishan City, Wuzhishan National Forest Park, 18°54'18"N 109°41'04"E, 800 m a.s.l., on leaves, 8 Sep. 2017, Wei C. Wang 139408 (HMAS-L).

DISTRIBUTION—Indonesia (Sérusiaux 1992) and China (Hainan).

COMMENTS—*Eremothecella macrosperma* is easily confused with *Arthonia palmulacea* due to their similar thallus and apothecia, but *E. macrosperma* has much larger ascospores with more numerous septa.

Pilocarpaceae Zahlbr.

Byssoloma annuum (Vain.) G. Thor, Lücking & Tat. Matsumoto,

Symb. Bot. Upsal. 32(3): 29 (2000)

FIG. 2C, D

= *Byssoloma dimerelloides* Sipman & Aptroot, Willdenowia
20: 240 (1991) [fide Thor & al. 2000]

= *Byssoloma clauzadei* Kalb & Vězda, Bull. Soc. Linn. Provence
45: 236 (1994) [fide Thor & al. 2000]

THALLUS continuous, smooth, greyish green. APOTHECIA pale yellowish brown, 0.3–0.6 mm diam, slightly constricted at the base, disc flat to convex, margin white, compact; excipulum inner part pale yellow, outer part colorless, composed of loosely hyphae, containing crystals; epihymenium indistinct; hymenium colorless, 50–60 µm tall; hypothecium pale yellow, containing crystals; apothecial base colorless. ASCI 8-spored; ascospores colorless, ellipsoid, 3-septate, without septal constrictions, 12–15 × 3 µm (n = 120).

CHEMISTRY—not tested.

SPECIMENS EXAMINED: CHINA. HAINAN, Ledong Co., Jianfeng Ridge, 18°44'N 109°10'E, 1000 m a.s.l., on leaves, 10 Sep. 2000, Man R. Huang 112833 (HMAS-L); 18°44'48"N 109°10'22"E, 900 m a.s.l., on leaves, 3 Apr. 1993, Yu M. Jiang & Shou Y. Guo 138621 (HMAS-L); Changjiang Co., Bawang Ridge, 19°15'31"N 109°02'54"E, 670 m a.s.l., on leaves, 20 May 2007, T. Zhang 126588, 126728, 126597, 126603 (HMAS-L); Wuzhishan City, Mt. Wuzhishan, 18°53'N 109°41'E, 900 m a.s.l., on leaves, 20 Apr. 2000, Jiang C. Wei 112938, 112809, 112823 (HMAS-L); 18°54'18"N 109°41'04"E, 800 m a.s.l., on leaves, 8 Sep. 2017, Wei C. Wang 139408 (HMAS-L).

DISTRIBUTION—Australia, Japan (Thor & al. 2000), Thailand (Papong & al. 2007), Philippines, Papua New Guinea (Aptroot & Sipman 1991), and China (Hainan).

COMMENTS—*Byssoloma annuum* is common in Asian tropical rainforests and has been reported several times in Asia. Its pale yellow apothecia easily distinguish it from other species. *Byssoloma carneum* and *B. sprucei* are closely related to it, but both have 3-septate ascospores, compact margins, and exciples with crystals; additionally, *B. sprucei* differs by its reddish brown apothecia, orange brown hypothecium, and brown apothecial base, and *B. carneum* differs

by farinose thallus dispersed into irregular patches, flat to slightly concave apothecia, and yellowish brown hypothecium (Lücking 2008).

Byssoloma subleucoblepharum G. Thor, Lücking & Tat. Matsumoto,

Symb. Bot. Upsal. 32(3): 31 (2000)

FIG. 2E, F

THALLUS thin, smooth, grey-green; prothallus not seen. APOTHECIA small, about 0.1 mm diam., rounded, grey-green, similar to the color of thallus, slightly convex, margin byssoid, composed of loose grey-white hyphae; epihymenium indistinct; hymenium colorless; apothecial base aeruginous. ASCI 8-spored; ascospores 3-septate, ellipsoid, colorless, $8\text{--}10 \times 2.5 \mu\text{m}$ ($n = 80$).

CHEMISTRY—not tested.

SPECIMENS EXAMINED: CHINA. HAINAN, Changjiang Co., Bawang Ridge, $19^{\circ}15'31''\text{N}$ $109^{\circ}02'54''\text{E}$, 700 m a.s.l., on leaves, 5 Oct. 2008, B. Gao 126436, 126449 (HMAS-L).

DISTRIBUTION—Japan (Thor & al. 2000) and China (Hainan).

COMMENTS—*Byssoloma subleucoblepharum* is small and hard to find. It is characterized by its small greyish green apothecia, aeruginous apothecial base, which distinguishes it from all other known *Byssoloma* species.

Gomphillaceae Walt. Watson

Calenia lueckingii C. Hartmann, Mycotaxon 59: 484 (1996)

FIG. 3A, B

THALLUS continuous, verrucose, green. APOTHECIA immersed-erumpent, pale green, 0.3–0.5 mm diam., disc smooth, slightly concave; epihymenium with epithelial algae; hymenium and hypothecium colorless. ASCI 1-spored; ascospores colorless, ellipsoid, muriform, large, $40\text{--}55 \times 20 \mu\text{m}$ ($n = 60$).

CHEMISTRY—not tested.

SPECIMENS EXAMINED: CHINA. HAINAN, Changjiang Co., Bawang Ridge, Baishitan scenic spot, $19^{\circ}07'07''\text{N}$ $109^{\circ}09'12''\text{E}$, 700 m a.s.l., on leaves, 4 Sep. 2017, Wei C. Wang 139419, 139417 (HMAS-L); Wuzhishan City, Wuzhishan National Forest Park, $18^{\circ}54'18''\text{N}$ $109^{\circ}41'04''\text{E}$, 800 m a.s.l., on leaves, 8 Sep. 2017, Wei C. Wang 139427, 139409, 139431, 139430, 139410, 139429, 139411, 139428 (HMAS-L); Mt. Wuzhishan, tropical rain forest scenic spot, $18^{\circ}51'59''\text{N}$ $109^{\circ}40'30''\text{E}$, 600 m a.s.l., on leaves, 9 Sep. 2017, Wei C. Wang 139414, 139413 (HMAS-L).

DISTRIBUTION—Neotropics (Lücking 2008) and China (Hainan; the first record from the paleotropics).

COMMENTS—Species of *Calenia* are not abundant on Hainan, with the exception of *C. lueckingii*, which is recognized by its pale green apothecia with epithelial algae, in addition to the pale green, verrucose thallus, and the single, large, muriform ascospores. Most similar are *Gyalectidium* species, which differ by their much smaller apothecia, often with a split between excipulum and thallus margin.

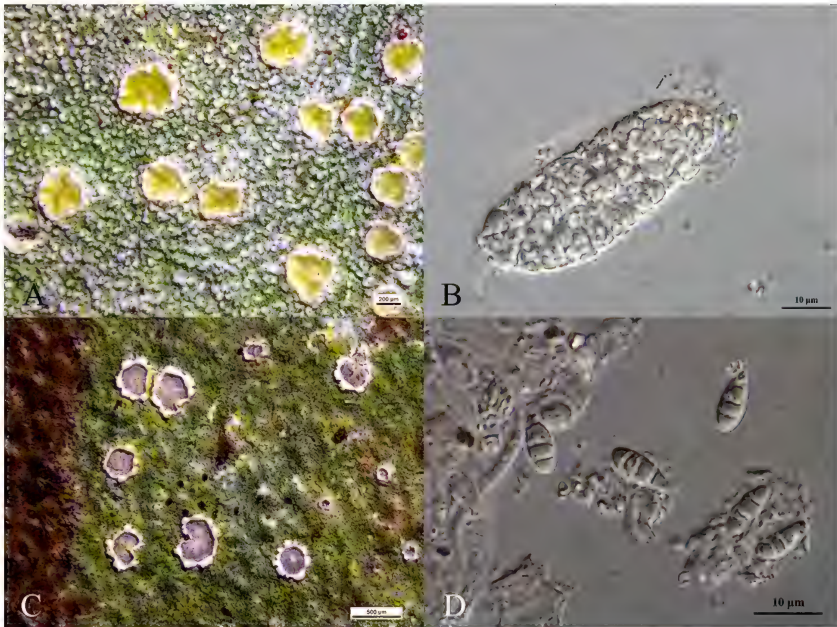


FIG. 3. Habitus and ascospores. A, B: *Calenia lueckingii* (HMAS-L Wang 139414); C, D: *Chroodiscus argillaceus* (HMAS-L Wang 139433). Scale bars: A = 200 µm; B, D = 10 µm; C = 500 µm.

Graphidaceae Dumort.

Chroodiscus argillaceus (Müll. Arg.) Lücking & Papong,

Bryologist 112: 154 (2009)

FIG. 3C, D

THALLUS continuous, greyish green, sometimes covered with yellow granules. APOTHECIA immersed-erumpent, disc flat, dark grey, 0.2–0.5 mm diam.; epihymenium indistinct; hymenium colorless, about 35 µm tall, consisted of parallel hyphae; hypothecium poorly developed, almost absent. ASCI 8-spored; ascospores colorless, ellipsoid, 3-septate, 6–10 × 3 µm (n = 60).

CHEMISTRY—not tested.

SPECIMENS EXAMINED: CHINA. HAINAN, Ledong Co., Jianfeng Ridge, Mingfeng Valley, 18°44'36"N 108°50'39"E, 960 m a.s.l., on leaves, 6 Sep. 2017, Wei C. Wang 139422, 139433, 139421 (HMAS-L); Wuzhishan City, Wuzhishan National Forest Park, 18°54'18"N 109°41'04"E, 800 m a.s.l., on leaves, 8 Sep. 2017, Wei C. Wang 139412 (HMAS-L).

DISTRIBUTION—Thailand (Papong & al. 2007) and China (Hainan).

COMMENTS—*Chroodiscus* has been studied in Southeast Asia by Papong & al. (2009). *Chroodiscus khaolungensis* resembles *C. argillaceus* due to 3-septate ascospores but differs in having longer (18–24 µm) ascospores.

Coenogoniaceae Stizenb.*Coenogonium minimum* (Müll. Arg.) Lücking,

Fl. Neotrop. Monogr. 103: 572 (2008)

FIG. 4A, B

THALLUS continuous, smooth, greyish green. APOTHECIA brown, 0.1–0.2 mm diam., disc strongly concave, margin persistent; excipulum well developed, colorless, paraplectenchymatous; epihymenium, hymenium and hypothecium colorless. ASCI 8-spored; ascospores colorless, ellipsoid, 1-septate, $10\text{--}14 \times 3 \mu\text{m}$ ($n = 120$).

CHEMISTRY—not tested.

SPECIMEN EXAMINED: CHINA. GUANGXI, Shangsi Co., Shiwandashan Forest Park, $21^{\circ}53'08''\text{N}$ $107^{\circ}54'41''\text{E}$, 657 m a.s.l., on leaves, 25 May 2015, Xin L. Wei & Jiao H. Wang 133031 (HMAS-L).

DISTRIBUTION—Neotropics (Lücking 2008), Vietnam (Nguyen & al. 2011), and China (Guangxi).

COMMENTS—*Coenogonium minimum* is characterized by its small brown apothecia with strongly concave discs. *Coenogonium lisowskii* and *C. dilucidum* are closely related to *C. minimum* in both having small apothecia, but

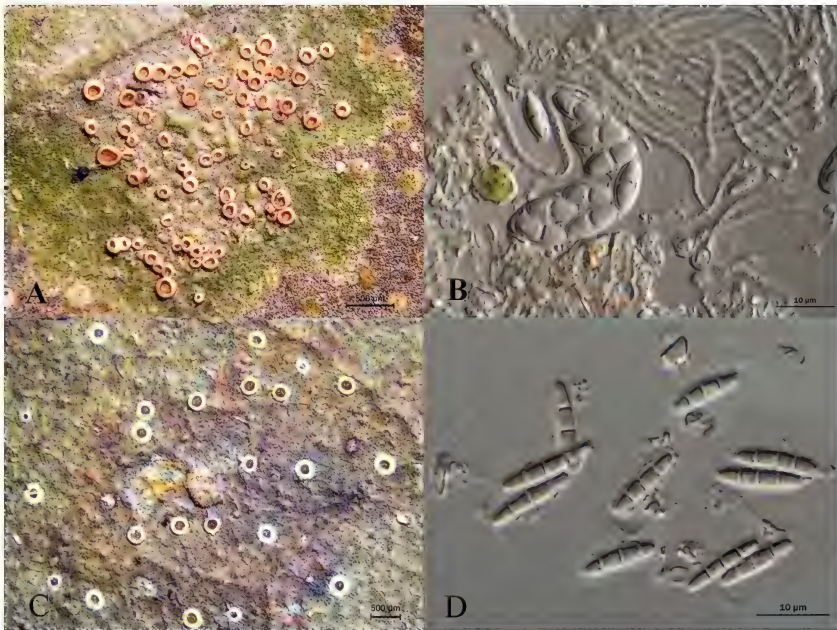


FIG. 4. Habitats and ascospores. A, B: *Coenogonium minimum* (HMAS-L Wei & Wang 133031); C, D: *Semigyalecta paradoxa* (HMAS-L Jiang & Guo 138622). Scale bars: A, C = 500 μm ; B, D = 10 μm .

C. lisowskii has wax-colored to yellow-brown apothecia and smaller ascospores, and *C. dilucidum* has wax-colored to pale yellow apothecia (Lücking 2008).

Gyalectaceae Stizenb.

Semigyalecta paradoxa Vain.,

Ann. Acad. Sci. Fenn., Ser. A 15(6): 153 (1921)

FIG. 4C, D

THALLUS continuous, smooth, greyish brown; prothallus black along the margin. APOTHECIA sessile, round, 0.2–0.4 mm diam., <80 µm high, slightly constricted at the base, disc flat, dark brown, margin well developed, smooth, white when young, turning yellow when mature. Excipulum well developed, colorless with pale yellow tinge, containing crystals; epihymenium indistinct; hymenium colorless, about 35 µm tall; hypothecium dark brown. ASCI 8-spored; ascospores fusiform, 3-septate, colorless, 10–15 × 2.5 µm (n = 60).

CHEMISTRY—not tested.

SPECIMENS EXAMINED: CHINA. HAINAN, Ledong Co., Jianfeng Ridge, 18°44'N 109°10'E, 900 m a.s.l., on leaves, 3 Apr. 1993, Yu M. Jiang & Shou Y. Guo 138608, 138622, 138625, 138640 (HMAS-L).

DISTRIBUTION—Japan (Thor & al. 2000), Philippines, Malesia (Santesson 1952), and China (Hainan).

COMMENTS—The monotypic genus *Semigyalecta* established by Vainio in 1921 was included in *Gyalectaceae*, but Santesson (1952) questioned this family placement. The genus is controversial, because its photobiont is difficult to identify; Kalb (1994) considered *Semigyalecta* to be a non-lichenized fungus, and Lücking did not reach a definite conclusion after studying the specimens from Japan (Thor & al. 2000). Our Hainan specimens had a photobiont in the thallus. The genus is obviously in need of further research to determine its appropriate classification status.

Acknowledgements

We thank Dr. Robert Lücking (Botanical Garden and Botanical Museum, Berlin, Germany) and Dr. André Aptroot (ABL Herbarium, Soest, The Netherlands) for presubmission reviews. This study was supported by the National Natural Science Foundation of China (31470149).

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***Hymenochaete cifuentesii*, *H. potosina*, and *H. raduloides* spp. nov. from the tropical dry forest of Mexico**

MAGDALENA CONTRERAS-PACHECO^{1,2}, RICARDO VALENZUELA²,
TANIA RAYMUNDO², LETICIA PACHECO^{1*}

¹ Depto. Biología, Universidad Autónoma Metropolitana-Iztapalapa,
Av. San Rafael Atlixco 186, Col. Vicentina, Ciudad de México, 09340, México

² Instituto Politécnico Nacional, Escuela Nacional de Ciencias Biológicas,
Plan de Ayala y Carpio s/n Col. Santo Tomas, México, D.F. 11340, México

* CORRESPONDENCE TO: pacheco@xanum.uam.mx

ABSTRACT—Three new species, *Hymenochaete cifuentesii*, *H. potosina*, and *H. raduloides*, are described and illustrated from Mexico. The specimens were collected in San Luis Potosí State in a tropical dry forest. Holotypes are deposited in ENCB Herbarium with isotypes in UAMIZ.

KEY WORDS—*Agaricomycetes*, fungal diversity, *Hymenochaetaceae*, taxonomy

Introduction

Hymenochaete Lév. is the type genus of *Hymenochaetaceae* Donk and *Hymenochaetales* Oberw. It includes annual to perennial species characterized by resupinate, effused-reflexed, or pileate (sessile and stipitate) basidiomata with papery, coriaceous, or woody contexts; smooth, warted, folded, cracked, or hydroid hymenophores with numerous setae; occasional crystals or granulations between or covering the hyphae; basidiomata brown often with reddish brown, yellowish, gold, or mustard hues; a color darkening in KOH (xanthocroic reaction); and a monomitric or pseudodimitic hyphal system with simple septa (Léger 1998, Parmasto 2001, He & Dai 2012, Parmasto & al. 2014). More than 130 species have been described in *Hymenochaete*, (one of the largest genera in *Hymenochaetaceae*), all of which cause white fibrous or pocket rot in dead wood (Parmasto 2001, Parmasto & al. 2014).

Twenty-one species have been reported from Mexico: *Hymenochaete allantospora* Parmasto, *H. americana* Gresl. & Parmasto, *H. carpatica* Pilát, *H. cervina* Berk & M.A. Curtis, *H. cinnamomea* (Pers.) Bres., *H. curtisii* (Berk.) Morgan, *H. damicornis* (Link) Lév., *H. epichlora* (Berk. & M.A. Curtis) Cooke, *H. episphaeria* (Schwein.) Massee, *H. escobarii* J.C. Léger, *H. digitata* Burt, *H. fulva* Burt, *H. leonina* Berk. & M.A. Curtis, *H. luteobadia* (Fr.) Höhn. & Litsch., *H. opaca* Burt, *H. pinnatifida* Burt, *H. resupinata* (Sw.) Parmasto, *H. rhabbarbarina* (Berk.) Cooke, *H. rheicolor* (Mont.) Lév., *H. rubiginosa* (Dicks.) Lév., and *H. unicolor* Berk. & M.A. Curtis (Welden & Lemke 1961; Reeves & Welden 1967; Marmolejo & al. 1981; Valenzuela & al. 1996; Léger 1998; Parmasto 2001; Pérez-Ramírez 2002, Montañón & al. 2006; Raymundo & al. 2009, 2013; Vázquez-Mendoza & Valenzuela 2010).

During taxonomic work in the tropical dry forest from State of San Luis Potosí, Mexico, several *Hymenochaete* specimens presented distinct morphotypes that could not be satisfactorily accommodated in any previously described species. Here we describe and illustrate three new *Hymenochaete* species associated with the tropical dry forest of Mexico.

Materials & methods

The specimens were collected in 2006, 2007, and 2009 from the tropical dry forest of the Río Verde municipality, Tamasopo, San Luis Potosí State, Mexico. They are deposited in the fungi collection “Dr. Gastón Guzmán Huerta” at the Herbarium of the Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, Mexico City, Mexico (ENCB) with duplicates in Herbarium, Universidad Autónoma Metropolitana–Iztapalapa, Mexico City, Mexico (UAMIZ). Longitudes and latitudes were obtained with a Garmin eTrex® GPS. Color codes follow the Munsell Color Company (M; 1954) soil color chart and Kornerup & Wanscher (KW; 1978). Morphological examinations followed protocols outlined by Léger (1998) and Parmasto (2001). Anatomical elements were measured in 5% aqueous KOH or Melzer’s reagent from rehydrated tissues. Macroscopic features were photographed with a Nikon D7000 camera, and microscopic elements with a Nikon Coolpix 9000 camera. Most terminology follows Ulloa & Hanlin (2012).

Taxonomy

Hymenochaete cifuenterisii Contr.-Pach., R. Valenz., Raymundo & Pacheco, **sp. nov.**

PLATES 1, 2, 7.A

MYCOBANK MB 824898

Differs from *Hymenochaete americana* by its shorter, ellipsoid to oblong spores; its abundant, lanceolate, slightly curved setae growing parallel to hyphae that deposit reddish granules into cambium cells; and its habitat in a tropical dry forest.

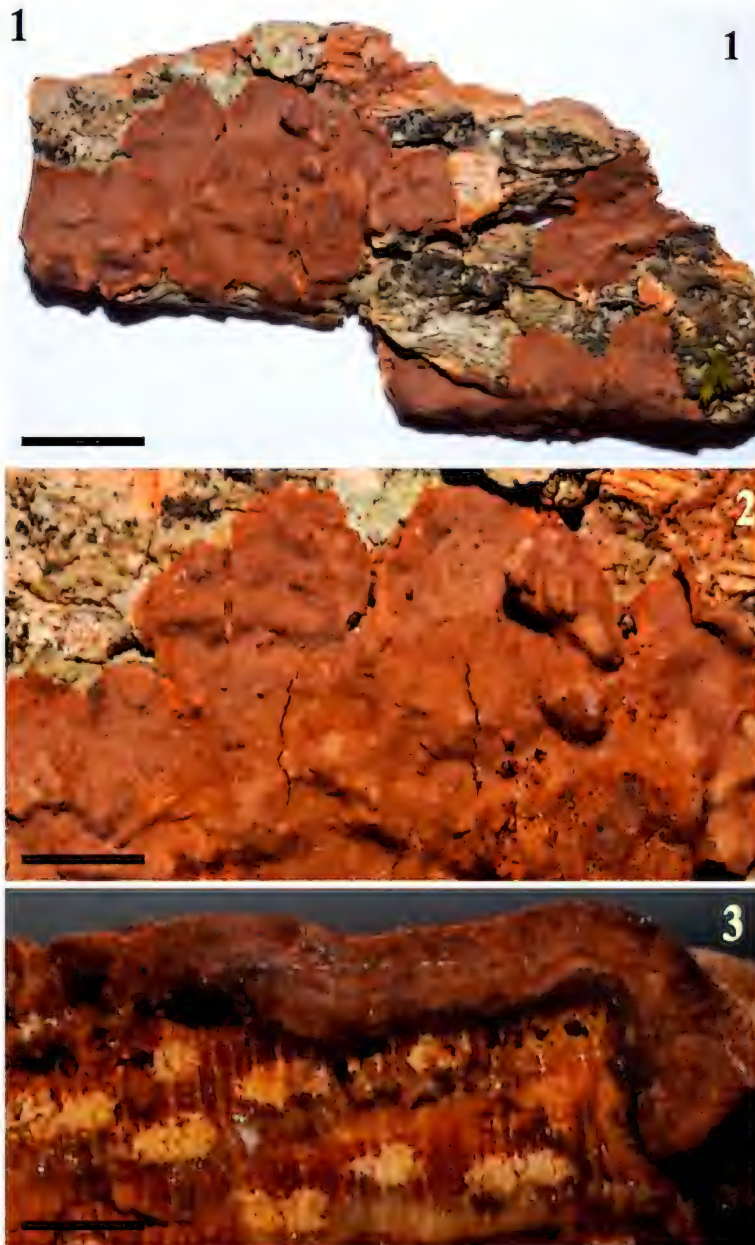


PLATE 1. *Hymenochaete cifuentesii* (holotype, ENCB: Raymundo & Valenzuela 1281).
1. Basidiome on substrate; 2. Margin of the basidiome; 3. Basidiome, cross-section.

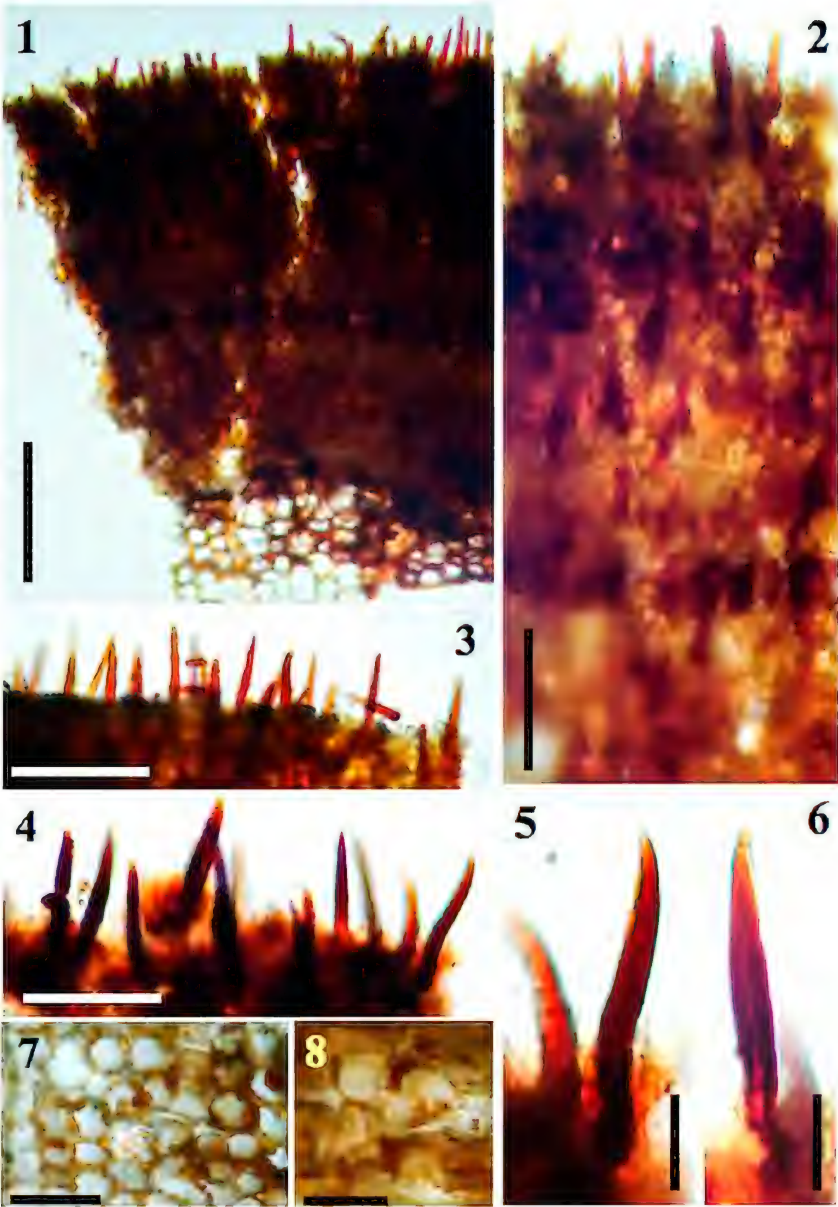


PLATE 2. *Hymenochaete cifuertesii* (holotype, ENCB: Raymundo & Valenzuela 1281).
1, 2. Basidiome, cross-section; 3–6. Setae; 7, 8. Plant substrate cambium cells showing granules.

TYPE: Mexico, San Luis Potosí State, municipality of Río Verde, km 70 Ciudad Valles–Río Verde road, 22°32'17"N 100°29'13"W, alt. 900 m, 26 November 2006, T. Raymundo & R. Valenzuela 1281 (**Holotype**, ENCB; **isotype**, UAMIZ).

ETYMOLOGY: In honor of our adviser, Professor Joaquín Cifuentes Blanco, a great Mexican mycologist.

BASIDIOME resupinate, closely adnate, corky, 57–97 × 20–30 mm diam, 0.4–0.5 mm thick, pale brown (M: 10YR 5/8; KW: 7E5). HYMENOPHORE smooth, with scattered tubercles, zonate, not cracked. Margin abrupt, sterile with setae, dark yellowish brown (M: 10YR 6/8; KW: 5E7). Cortex and tomentum absent. HYMENIUM 47–57 µm thick, subiculum <560 µm thick, with numerous stratified and parallel aligned setae and hyphae. HYPHAL SYSTEM monomitic, generative hyphae of two types: a) in subhymenium and subiculum hyaline, 1–1.5 µm diam, simple septa; b) in subiculum with slightly thickened (<1 µm) walls, reddish brown to dark brown in KOH, 1.6–3.2 µm diam, simple septa; subiculum hyphae growing into the bark to deposit reddish granules within the cambium cells. SETAE 52–110 × 6–15 µm, lanceolate, slightly curved, abundant, reddish brown to dark brown in KOH, projecting <30 µm; the acute tip encrusted with crystals and the base with hyphae interwoven compactly. BASIDIA 20–28 × 4–6.4 µm, subclavate, tetrasporic, sterigmata 2.4–4 µm long. BASIDIOSPORES 5–7.6 × 2.4–4 µm, ellipsoid to oblong, hyaline, thin-walled, smooth.

ECOLOGY AND DISTRIBUTION—*Hymenochaete cifuentesii* grows gregariously on fallen and dead angiosperm twigs in tropical dry forest and causes a pocket rot. Known only from the type locality.

ADDITIONAL SPECIMEN EXAMINED—MEXICO. SAN LUIS POTOSÍ, municipality of Río Verde, km 70 Ciudad Valle–Río Verde road, 1 November 2007, T. Raymundo & R. Valenzuela 1276 (ENCB).

COMMENTS—*Hymenochaete cifuentesii* belongs to *Hymenochaete* sect. *Fulthochaete*, which Léger (1998) characterizes as species having a setal layer and lacking a cortex and tomentum; Our new species, which differs from other species in the section by its parallel orientation of hyphae and setae is similar to *H. americana*, which is distinguished by its chocolate brown color and longer, cylindrical to slightly curved (almost sigmoid) spores (7.5–9.2 × 2.5–3 µm; Parmasto 2001). *Hymenochaete livens* Bres. differs by its light brown color, and its wider setae (60–100 × 12–20 µm; Léger 1998). *Hymenochaete spreta* Peck [= *H. cinnamomea* subsp. *spreti* (Peck) Parmasto] differs by its numerous longer (70–120 × 5–9 µm) non-incrusted setae and smaller (4.5–6.5(7) × 1.8–2.8(3.2) µm) spores (Parmasto 2001, as *H. cinnamomea* subsp. *spreti*).

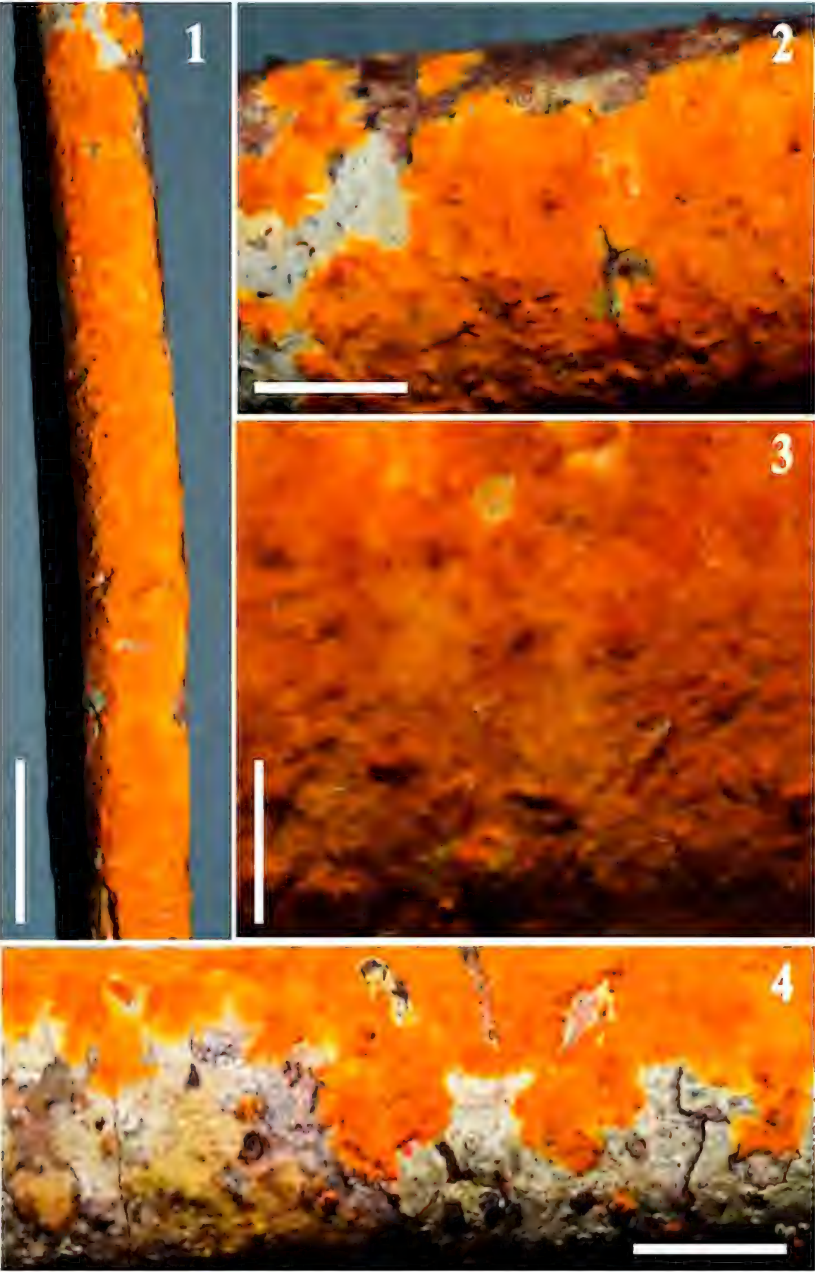


PLATE 3. *Hymenochaete potosina* (holotype, ENCB: Valenzuela 13381). 1. Resupinate basidiome; 2. Basidiome on substrate; 3. Hymenophore; 4. Basidiome margin.

Hymenochaete potosina Contr.-Pach., R. Valenz., Raymundo & Pacheco,
sp. nov.

PLATES 3, 4, 7B

MYCOBANK MB 824899

Differs from *Hymenochaete epichlora* by its smooth basidiome adhered to the substrate, its larger setae with crystals, its production of two types of generative hyphae, its bigger ellipsoid basidiospores, and by its habitat in a tropical dry forest.

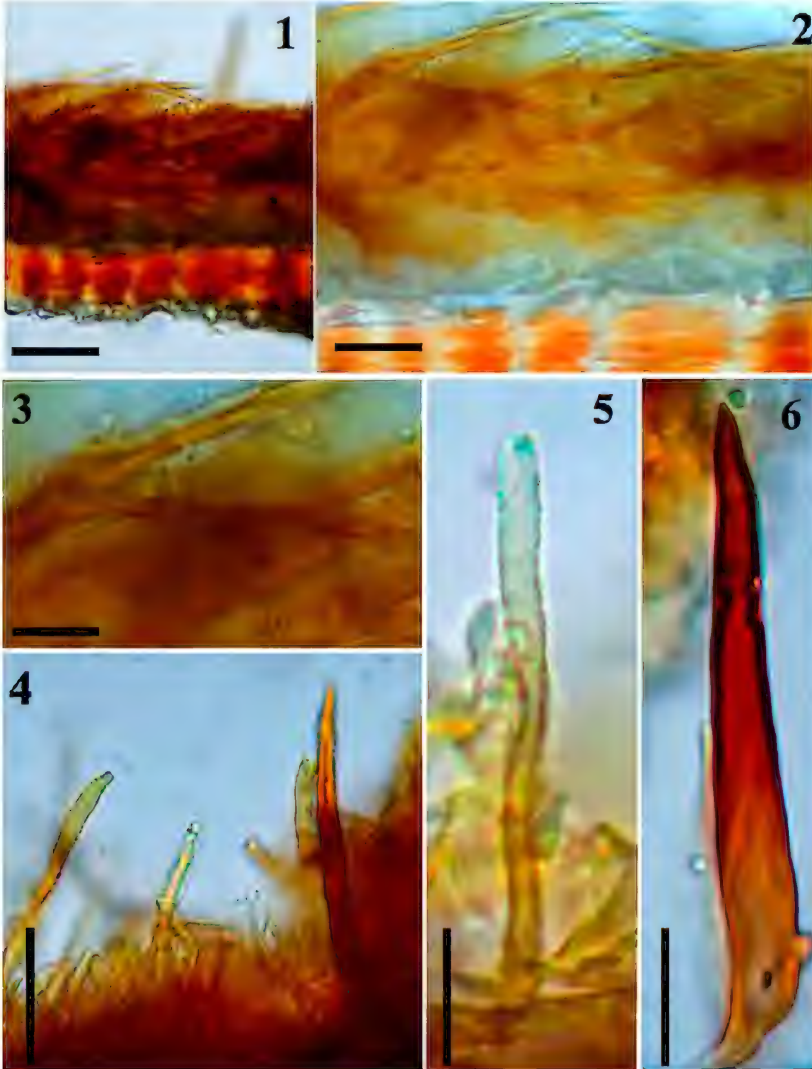


PLATE 4. *Hymenochaete potosina* (holotype, ENCB: Valenzuela 13381). 1, 2. Basidiome, cross-section; 3. Hyphal system; 4. Hymenium; 5. Hyphae with some crystals; 6. Setae.

TYPE: Mexico, San Luis Potosí State: municipality of Río Verde, km 64–65 Ciudad Valles–Río Verde road, 22°52'51"N 99°26'18"W, alt. 630 m, 30 August 2009, R. Valenzuela 13381 (Holotype, ENCB; isotype, UAMIZ).

ETYMOLOGY: The specific epithet is a reference to the type locality: San Luis Potosí.

BASIDIOME resupinate, crustose, adnate, cottony, 150–250 µm thick, 65 × 10 mm, then confluent, strong orange (M: 5YR 6/8, 7/8; KW: 6A8, 6A6). HYMENOPHORE smooth, azonate. Margin determinate, byssoid, yellowish (M: 7.5Y 9/4; KW: 4A7), sterile with setae. Cortex and tomentum absent. HYMENIUM 76 µm thick, subiculum a 57 µm thick layer composed of intermingled hyphae and setae. HYPHAL SYSTEM monomitic, generative hyphae with simple septa of two types: a) yellowish to yellowish brown, 3–4 µm diam, with slightly thickened (1 µm) walls and crystals sometime present; b) hyaline, 2–4 µm diam, with crystals, projecting above hymenium. SETAE 104–120 × 3.2–5.6 µm, subulate, scattered, brownish, with blunt tip, incrusted with crystals, projecting above hymenium 50–70 µm. BASIDIA 21.6 × 3.2 µm, subclavate, tetrasporic, sterigmata 1.6 µm long. BASIDIOSPORES 5.6–7 × 2.4–3 µm, ellipsoid, hyaline, thin-walled, smooth.

ECOLOGY AND DISTRIBUTION—Our specimen was collected in the tropical dry forest. The species is known only from the type locality.

COMMENTS—*Hymenochaete potosina* belongs to *Hymenochaete* sect. *Fultochaete* based on the setal layer and context (Léger 1998). Our new species is characterized by its smooth (not cracked) resupinate basidiome with a smooth hymenophore, strong orange color, absence of cystidia, and yellowish crystalline generative hyphae that project above the hymenium. *Hymenochaete potosina* differs from *H. epichlora*, which is distinguished by smaller basidiospores (4–5 × 2–2.5 µm) and setae (35–55 × 5–6.5 µm, without crystals; Léger 1998).

Hymenochaete raduloides Contr.-Pach., R. Valenz., Raymundo & Pacheco, **sp. nov.**

PLATES 5, 6, 7.C

MYCOBANK MB 824900

Differs from other *Hymenochaete* species by the combination of its resupinate basidiome, its hymenophore with well-defined teeth, its pseudodimitic hyphal system pseudodimitic, the absence of a black zone next to substratum, and its habitat in a tropical dry forest.

TYPE: Mexico, San Luis Potosí State, municipality of Río Verde, km 70 Ciudad Valles–Río Verde road, 22°32'17" 100°29'13", alt. 900 m, 1 November 2007, T. Raymundo 1910 (Holotype, ENCB; isotype, UAMIZ).

ETYMOLOGY: The specific epithet refers to a hymenophore with flattened teeth.

BASIDIOME resupinate, closely adnate, corky, 200–400 µm thick, 120 × 28 mm diam, as round patches, confluent, reddish brown (M: 7.5YR 6/10; KW: 5B8).

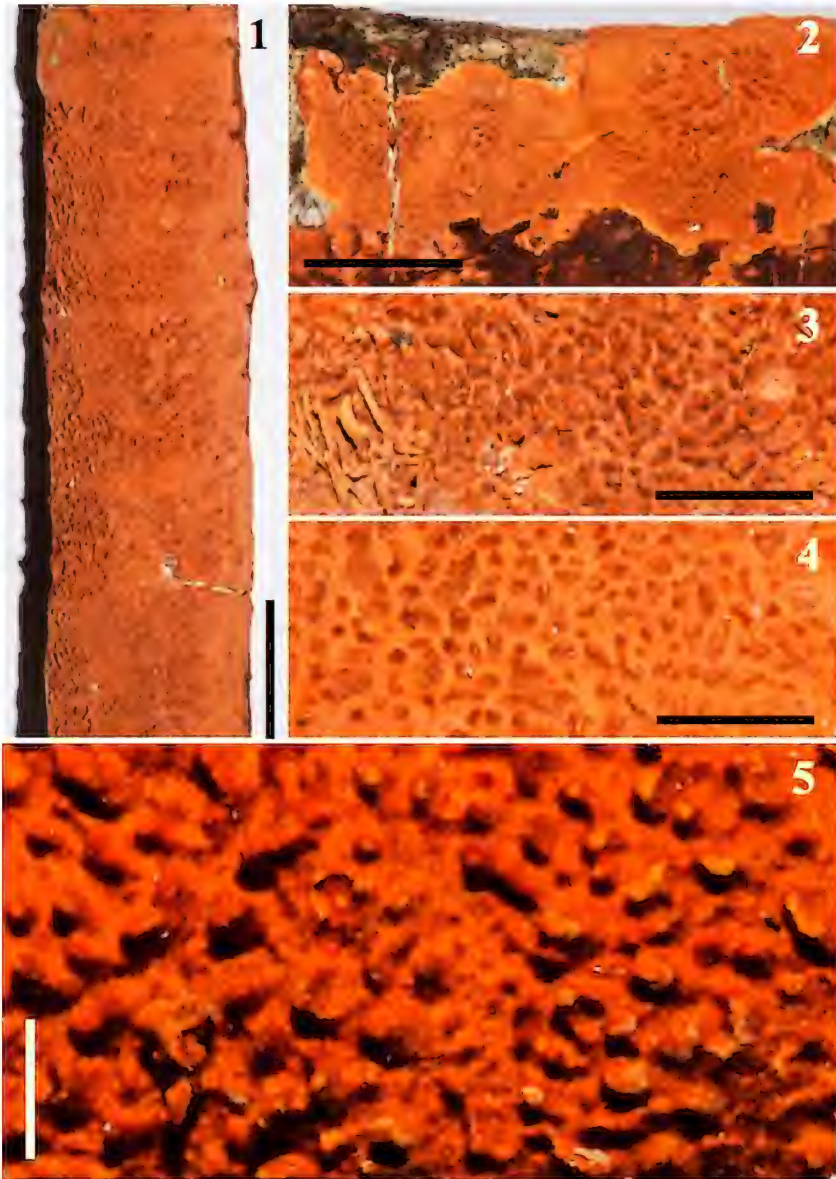


PLATE 5. *Hymenochaete raduloides* (holotype, ENCB: Raymundo 1910). 1. Resupinate basidiome; 2. Basidiome on substrate; 3 Hydroid hymenophore; 4. Teeth of margin; 5. Teeth and setae, magnified.

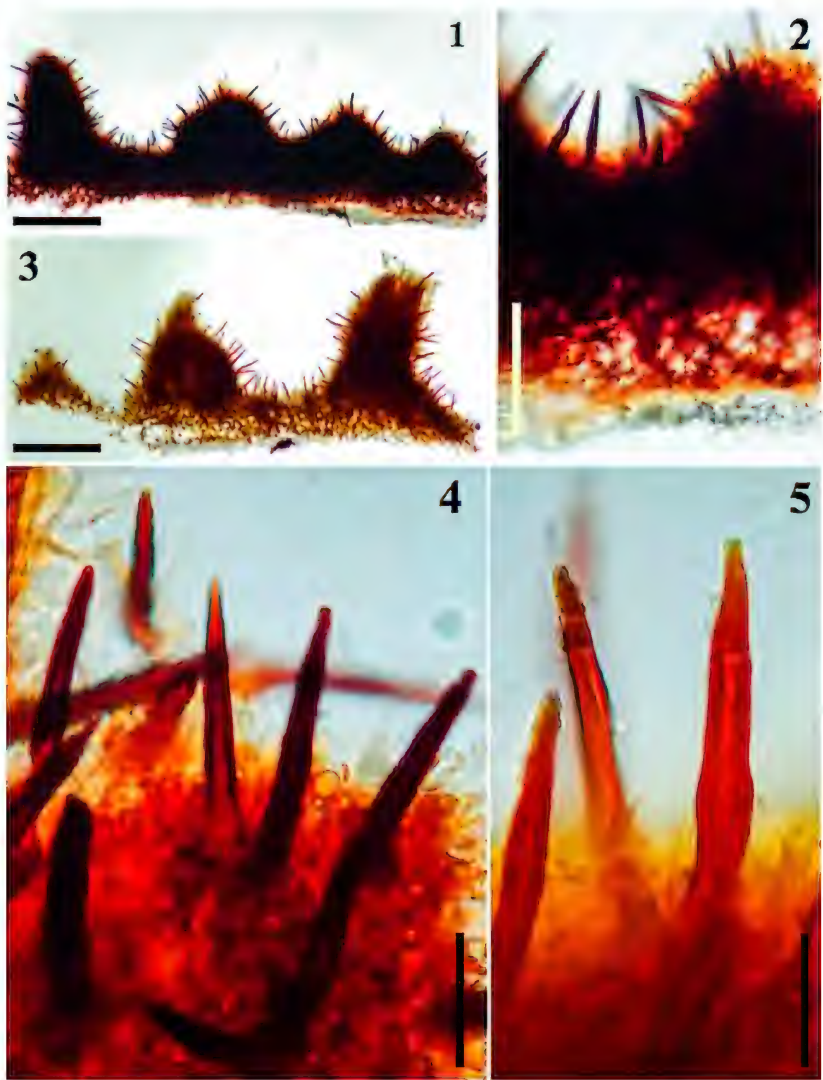


PLATE 6. *Hymenochaete raduloides* (holotype, ENCB: Raymundo 1910). 1–3. Basidiome, cross-section; 4, 5. Setae with crystals.

HYMENOPHORE hydroid to irpicoid, with round to flattened teeth (2–3 mm), sometimes fused in basal parts, with an obtuse to acute tip, <1 mm long, with numerous setae. Margin determinate, abrupt, fibrillose, with setae, sometimes lighter coloured, yellowish (7.5Y 9/4, 5A4). Cortex and tomentum absent.

HYMENIUM 40–80 μm thick, subiculum 160–320 μm thick comprising the hyphal layer and lacking a dark line above the hymenium. HYPHAL SYSTEM pseudodimitic, with two hyphal types with simple septa intermingled: a) brown, 3–4 μm diam, walls 0.8 μm thick, incrustated with crystals; b) hyphae hyaline, 2–4 μm diam, thin-walled. SETAE abundant throughout the hymenium, 76–119 $\times$ 7.2–8.8 μm , subulate, vacuolate, brownish, acute. BASIDIA 8–12 $\times$ 2.4–3.2 μm , subclavate, tetrasporic, sterigmata 2.4–3.2 μm long. BASIDIOSPORES 4–5 $\times$ 1.6–3.2 μm , ellipsoid, hyaline, thin walled, smooth.

ECOLOGY AND DISTRIBUTION—Our specimen was collected in dry forest, on dead wood. The species is known only from the type locality.

COMMENTS—This specimen belongs to *Hymenochaete* sect. *Gymnochaete* based on its possession of a setal layer (Léger 1998). It is characterized by a hydroid hymenophore, spores 4–5 $\times$ 1.6–3.2 μm , abundant hymenial setae incrustated with crystals, and a pseudodimitic hyphal system characterized by two sets of hyphae with simple septa: one thin-walled and hyaline, the other thick-walled and brown. Both *H. raduloides* and *H. odontoides* S.H. He & Y.C. Dai [= *Hydnochaete tabacina* (Berk. & M.A. Curtis ex Fr.) Ryvarden] produce a hymenophore with well-developed teeth; however, *H. odontoides* is distinguished by producing cortex and tomentum, and by its longer, narrower, allantoid to cylindrical spores (5–6 $\times$ 1–1.5 μm ; Ryvarden 1982, as *Hydnochaete tabacina*; Parmasto 2001, as *H. tabacina*).

Key to species of *Hymenochaete* in Mexico

1. Hymenophore hydroid or odontoid 2
1. Hymenophore smooth or with scattered tubercles 3
2. Tomentum absent, tissues pseudodimitic *H. raduloides*
2. Tomentum present, tissues monomitic, [dichohyphae & setae
(90–160 $\times$ 7.2–12 μm), numerous] *H. resupinata*
3. Basidiome pileate, effused-reflexed, or umbonate 4
3. Basidiome effused, margins sometimes slightly elevated 5
4. Basidiome pileate, sometimes branched [usually growing near trees] *H. damicornis*
4. Basidiome pileate without a stipe 7
5. Accessory elements present 6
5. Accessory elements absent 12
6. Dendrohyphidia with thickened walls, without tomentum [with cortex and hyphal
layer, sometime stratose, hyphal and setal layers alternating] *H. pinnatifida*
6. Pseudoacanthophyses numerous, with tomentum, [with cortex and hyphal layer;
two types (small and large) of setae present] *H. digitata*

7. Pileus flexible, hyphal layer well developed with loosely arranged hyphae, spores cylindrical, slightly curved, 1.5–2.5 µm diam	8
7. Pileus coriaceous, without hyphal layer or when present densely arranged, spores cylindrical to ellipsoid, 1.2–4.8 µm diam	9
8. Basidiome effused-reflexed, setae rare in sterile hymenium, hyphidia with coiled tips, spores 5.5–8.3 µm long	<i>H. curtisii</i>
8. Basidiome sessile-pileate or sessile-umbonate, setae rare or numerous, hyphidia absent, spores 4.5–7 µm long	<i>H. rheicolor</i>
9. Setae 4–8 µm diam	10
9. Setae 7–15 µm diam	11
10. Dendrohyphidia present, layer setal stratose [spores ellipsoid, 5–6.5 × 3.5–4.5 µm]	<i>H. escobarii</i>
10. Dendrohyphidia absent, setae 40–60 × 5–8 µm, [hyphidia numerous, with encrusted thickened walls]	<i>H. luteobadia</i>
11. Basidiome woody, pileal surface velutinous, setae 35–85 × 5–7 µm, spores ellipsoid with one side flattened, 4–5.1 × 2–3 µm	<i>H. rubiginosa</i>
11. Basidiome coriaceous, pileal surface hirsute, setae 80–150 × 10–15 µm, spores allantoid, 8.5–11 × 2–2.7 µm [hymenium not cracked] ...	<i>H. allantospora</i>
12. Hyphal layer present (sometimes thin)	13
12. Hyphal layer absent (only setal layer present)	18
13. Hyphal layer duplex[basidiome bisected by dark line, basidioles in a sterile hymenium]	<i>H. leonina</i>
13. Hyphal layer simple	14
14. Cortex present	15
14. Cortex absent	16
15. Setae incrusted, 70–100 × 8–12 µm, spores cylindrical or sigmoid, 7.5–9 × 2.5–3 µm	<i>H. americana</i>
15. Setae not incrusted, 70–90 × 7–9 µm, spores ellipsoid, 5–6 × 3–4 µm	<i>H. fulva</i>
16. Setae not encrusted (30–60 × 5–7 µm) [spores ellipsoid, 4.5–5.5 × 3–3.5 µm]	<i>H. unicolor</i>
16. Setae encrusted	17
17. Setae 30–60 × 5.5–9 µm, spores 3.5–5 × 1.8–2.5 µm	<i>H. epichlora</i>
17. Setae 60–105 × 5–8 µm, spores 4.5–5 × 2.5–3.5 µm	<i>H. rhabarbarina</i>
17. Setae 104–120 × 3.2–5.6 µm, spores 5.6 × 2.4 µm, ...	<i>H. potosina</i>
18. Basidiome stratose	19
18. Basidiome not stratose	20
19. Setae acicular	<i>H. cifuentesii</i>
19. Setae not acicular	<i>H. cinnamomea</i>
20. Setal tip obtuse, with crystals [hyphidia absent, cortex 30–40 µm, basidiome yellowish brown]	<i>H. episphaeria</i>
20. Setal tip acute	21

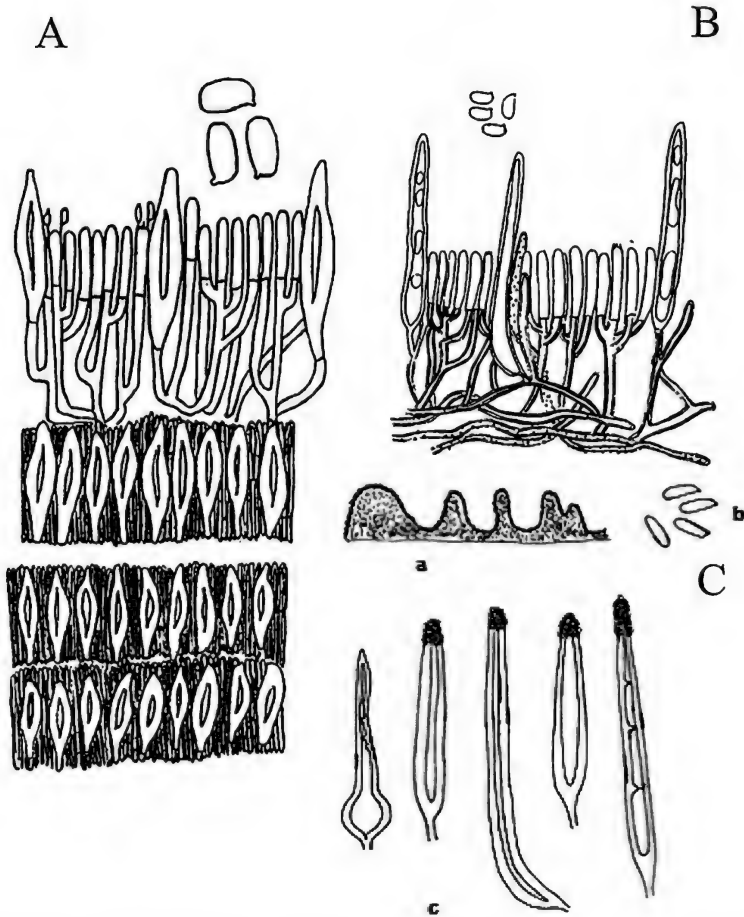


PLATE 7. A. *Hymenochaete: cifuentesii*: vertical section of basidiome. B. *Hymenochaete potosina*: basidiome, vertical section. C. *Hymenochaete raduloides*: a. basidiome, cross-section; b. basidiospore; c. setae.

- 21 Setae with crystals at apex [hyphidia present or absent,
hymenium smooth, spores ellipsoid $5-7.5 \times 3.5-4 \mu\text{m}$] *H. cervina*
21. Setae without crystals at apex 22
22. Spores cylindrical to subballantoid, $5-7.5 \times 3.5-4 \mu\text{m}$
[basidioles present, hymenial surface velutinous] *H. opaca*
22. Spores ellipsoid, $5.5-6.5 \times 3-3.5 \mu\text{m}$
[setae numerous, $50-90 \times 6-10 \mu\text{m}$]..... *H. carpatica*

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***Pestalotiopsis lijiangensis* sp. nov., a new endophytic fungus from Yunnan, China**

YING-KE ZHOU^{1, 2}, FU-PING LI², CHENG-LIN HOU^{1*}

¹ College of Life Science, Capital Normal University,
Xisanhuanbeilu 105, Haidian, Beijing 100048, PR China

² College of Mining Engineering, North China University of Science & Technology,
Bohai road 21, Caofeidianxincheng, Tangshan 063210, PR China

* CORRESPONDENCE TO: houchenglincn@yahoo.com

ABSTRACT—A new endophytic fungus, *Pestalotiopsis lijiangensis* from leaves of *Castanopsis carlesii* var. *spinulosa*, is described and illustrated. The new species is similar to *P. montellica* and *P. jesteri* in having lateral appendages at the upper conidial septum, but *P. lijiangensis* differs from both *P. montellica* and *P. jesteri* by its wider conidia with deeply pigmented doliform median cells and additionally from *P. jesteri* by the production of apical conidial appendages. A phylogenetic analysis of the combined sequence data of internal transcribed spacer (ITS), partial β -tubulin (TUB), and partial translation elongation factor1- α (TEF) supports *P. lijiangensis* on an independent branch within *Pestalotiopsis*.

KEY WORDS—*Amphisphaeriales*, coelomycetes, molecular phylogenetics, *Pestalotiopsidaceae*, taxonomy

Introduction

Pestalotiopsis Steyaert is characterized by relatively fusiform conidia, each comprising a hyaline basal cell, three pigmented median cells, and a hyaline apical cell with two or more apical appendages (Steyaert 1949). The interspecific delineation of *Pestalotiopsis* is based on conidial morphology (Guba 1961, Nag Raj 1993), conidiogenesis (Sutton 1980), and teleomorph association (Barr 1975, 1990; Zhu & al. 1991; Metz & al. 2000). The use of molecular data in resolving *Pestalotiopsis* species has been reviewed by Hu & al. (2007), Tejesvi & al. (2007), Liu & al. (2010), and Maharachchikumbura & al. (2011). These studies suggest that multi-locus phylogenetic analysis is needed to resolve the

cryptic species in the genus. A combination of internal transcribed spacer (ITS), partial β -tubulin (TUB), and partial translation elongation factor1- α (TEF) gene data gave the best resolution when compared to single gene analysis (Maharachchikumbura & al. 2012). Based on these analyses, *Pestalotiopsis* sensu lato has been divided into three genera: *Pestalotiopsis* sensu stricto, *Neopestalotiopsis* Maharachch. & al., and *Pseudopestalotiopsis* Maharachch. & al., after morphology and sequence data (ITS, TUB, and TEF) were combined to identify new cryptic species (Maharachchikumbura & al. 2014).

Here, we isolated two endophytic fungal strains from a leaf of *Castanopsis carlesii* var. *spinulosa* W.C. Cheng & C.S. Chao (*Fagaceae*); both strains shared the character of lateral appendages at the upper conidial septum with *P. montellica* (Sacc. & Voglino) Tak. Kobay. and *P. jesteri* Strobel & al. (nom. inval.) but differed from those species by the size and pigmentation of their conidia. We therefore propose this endophytic fungus as a new species, *Pestalotiopsis lijiangensis*. Its phylogenetic position was inferred from the combined sequences of ITS, TUB, and TEF.

Materials & methods

Observation of morphological characters

Conidia were produced in a monoculture derived from a single conidium that was selected for morphological observation. Morphological characters for identification were measured and photographed under an Olympus Bx51 light microscope. These characters included conidial length and width, appendage length and number, and the pigmentation of the three median conidial cells. Microscopic examination performed 30 repetitions.

DNA extraction, amplification, sequencing

Total DNA was extracted from fresh cultures following the protocol of Guo & al. (2000). For nucleotide sequence comparisons, nrLSU, ITS, TUB and TEF genes were amplified using primer pairs LR0R/LR5 (Vilgalys & Hester 1990, Rehner & Samuels 1994), ITS5/ITS4 (White & al. 1990), T1/Bt-2b (Glass & Donaldson 1995, O'Donnell & Cigelnik 1997), and EF1-728F/EF-2 (O'Donnell & al. 1998, Carbone & Kohn 1999), respectively. Amplification conditions for nrLSU, ITS and TEF followed Crous & al. (2012) and for TUB followed Lee & al. (2004). The PCR products were purified and edited by Zhongkexilin Biotechnology Co. Ltd. (Beijing, China). The new sequences were submitted to the GenBank database. Other sequences in this study were downloaded from GenBank (TABLE 1).

Phylogenetic analyses

The sequences were aligned with Clustal X1.81 (Thompson & al. 1997) and the results were adjusted manually where necessary to maximize alignment. All sites

TABLE 1. *Neopestalotiopsis* and *Pestalotiopsis* sequences used in the molecular analysis

SPECIES & VOUCHER	GENBANK ACCESSION No.		
	ITS	TUB	TEF
<i>N. saprophytica</i> MFLUCC12-0282	JX398982	JX399017	JX399048
<i>P. camelliae</i> CBS443.62	KM199336	KM199424	KM199512
<i>P. clavata</i> MFLUCC12-0268	JX398990	JX399025	JX39905
<i>P. cocculi</i> HHL-BZ	KM535703	KM573239	—
<i>P. colombiensis</i> CBS118553	KM199307	KM199421	KM199488
<i>P. disseminata</i> EC3A	EF055196	EF055233	—
<i>P. diversiseta</i> MFLUCC12-0287	JX399009	JX399040	JX399073
<i>P. gracilis</i> QQ-OZ	HM573281	HM535746	—
<i>P. hawaiiensis</i> CBS114491	KM199339	KM199428	KM199514
<i>P. intermedia</i> MFLUCC12-0259	JX398993	JX399028	JX399059
<i>P. jesteri</i> CBS109350	KM199380	KM199468	KM199554
“ <i>P. jesteri</i> ” MFLUCC12-0279	JX399012	JX399043	JX399076
<i>P. knightiae</i> CBS114138	KM199310	KM199411	KM199482
<i>P. lijiangensis</i> CFCC50738 [T]	KU860520	KU844184	KU844185
<i>P. lijiangensis</i> CFCC50739	MH8808834	MH880835	MH880836
<i>P. malayana</i> CBS102220	KM199306	KM199411	KM199482
<i>P. microspora</i> P20-02	HQ315843	—	—
<i>P. neglecta</i> QQ-TKZ	HM535758	HM573293	—
<i>P. olivacea</i> SY17A	EF055215	EF055251	—
<i>P. portugallica</i> CBS393.48	KM199335	KM199463	—
<i>P. rosea</i> MFLUCC12-0258	JX399005	JX399036	JX399069
<i>P. verruculosa</i> MFLUCC12-0274	JX398996	—	JX399061
<i>P. vismiae</i> HHL-DG	HM535704	HM573246	—

were treated as unordered and unweighted, and gaps were treated as missing data in the phylogenetic analyses. Firstly, new species in *Pestalotiopsis* sensu stricto, *Neopestalotiopsis*, and *Pseudopestalotiopsis* was estimated by the nrLSU sequence data. The result grouped *P. lijiangensis* in *Pestalotiopsis* sensu stricto (not shown). Analysis of a combined alignment dataset (ITS, TUB, and TEF) confirmed the relationship of the new species and other congeneric species in *Pestalotiopsis*. The alignment data was subsequently used for maximum parsimony (MP) analysis, in which a search for most parsimonious trees was conducted with the heuristic search algorithm with tree-bisection-reconnection (TBR) branch swapping in PAUP 4.0b10 (Swofford 2003). For each search, 1000 replicates of random stepwise sequence addition were performed, and all trees were saved per-replicate. The strength of the internal branches of the trees was tested with bootstrap analyses

using 1000 replications with the same search settings. For the Bayesian analysis, MrModeltest 3.7 with the Akaike information criterion was used to choose the substitution model for the separate dataset (Nylander & al. 2004), and the analysis was performed with MrBayes 3.1.2 (Huelsenbeck & Ronquist 2001, Ronquist & Huelsenbeck 2003). The detailed methods and parameters of MP and Bayesian analyses followed Maharachchikumbura & al. (2014). Bayesian posterior probabilities (PP) were obtained from the 70% majority rule consensus of the trees kept. Bootstrap percentages (BP) of more than 70 from 1000 replications are shown above the respective branches (FIG. 2). If >95% of the sampled trees were contained in a given clade, it was considered to be significantly supported by the data.

Taxonomy

Pestalotiopsis lijiangensis Y.K. Zhou & C.L. Hou, **sp. nov.**

FIG. 1

MYCOBANK MB 816081

Differs from *Pestalotiopsis montellica* by its wider conidia with deeply pigmented doliform median cells; and from *P. jesteri* by its wider conidia with deeply pigmented doliform median cells and by producing apical conidial appendages.

TYPE: China, Yunnan, Lijiang, alt. ca. 3800 m, endophytic in living leaves of *Castanopsis carlesii* var. *spinulosa*, 18 June 2014, Y.K. Zhou. **Holotype**, BJTC 1181; ex-type culture, CFCC50738; GenBank KU860520, KU844184, KU844185.

ETYMOLOGY: *lijiangensis* referring to the city where this species was obtained.

CONIDIOMATA pycnidia in culture on PDA, globose, scattered, semi-immersed, black, <100–300 µm diam; exuding globose, dark brown to black conidial masses. CONIDIOPHORES sparsely septate at base, branched or unbranched, subcylindrical, hyaline, smooth, <15 µm or reduced to conidiogenous cells. CONIDIOGENOUS CELLS discrete, lageniform to subcylindrical, hyaline, smooth, 10–25 × 2–5 µm ($x = 13.5 \times 3.7$ µm), proliferating 1–3 times percurrently. CONIDIA fusiform, 4-septate, 22–25 × 10–12 µm ($x = 23.8 \times 11.5$ µm), mean conidial length/width ratio = 2.96; bearing appendages; basal cell narrowly obconic with a truncate base bearing minute marginal frills, hyaline, smooth, 2–4 µm ($x = 3.4$ µm); three median cells doliform, brown, concolourous, walls verruculose and slightly constricted at the septa, together 14.5–18 µm ($x = 16$ µm), second cell from base 4.5–5.5 µm ($x = 5.3$ µm); third cell 4–5.1 µm ($x = 4.4$ µm); fourth cell 6–7.4 µm ($x = 6.3$ µm); apical cell obconic with an acute apex, hyaline, smooth, 2–3.5 µm ($x = 2.8$ µm). APPENDAGES tubular, unbranched, flexuous, more or less attenuated; apical appendage single, 3–8 µm ($x = 6.7$ µm); lateral appendages 2–4 (mostly 3), arising just above the septum separating the apical cell and subapical cell, unbranched 14–25 µm ($x = 18.6$ µm); basal appendage (occasionally absent) unbranched, attenuated, flexuous, centric 2–6 µm ($x = 4.8$ µm).

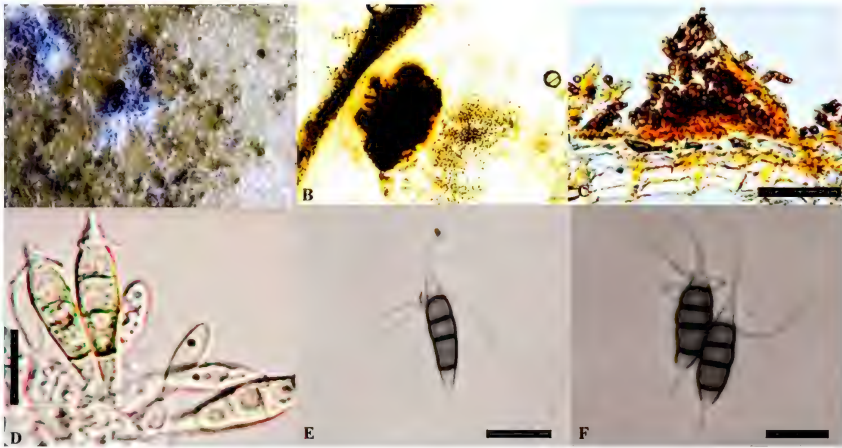


FIG. 1 *Pestalotiopsis lijiangensis* (CFCC50738): A. Conidiomata on PDA; B. Conidioma sporulating on PDA; C. Section of conidioma, split irregularly; D. Conidiophores and immature conidia; E, F. Conidia. Scale bars: C = 50 μ m; D = 30 μ m; E, F = 20 μ m.

ADDITIONAL MATERIAL EXAMINED: CHINA, YUNNAN, Lijiang, alt. ca. 3800 m, endophytic in living leaves of *Castanopsis carlesii* var. *spinulosa*, 18 June 2014, Y.K. Zhou (BJTC 1182, CFCC 50739; GenBank MH880834, MH880835, MH880836).

Phylogeny

To clarify species boundaries within *Pestalotiopsis*, a combined alignment of ITS, TUB, and TEF was created, containing 23 sequences (including the outgroup *Neopestalotiopsis saprophytica*) and 1519 characters. Of the 1519 characters (ITS = 552, TUB = 463, and TEF = 504), 890 were constant, 250 variable characters parsimony uninformative, and 379 characters parsimony informative. The MP analysis of sequences resulted in one most parsimonious tree with a length (TL) of 1738 steps, consistency index (CI) of 0.636, retention index (RI) of 0.708, homoplasy index (HI) of 0.604, and rescaled consistency index (RC) of 0.587. The Bayesian analysis resulted in a tree with the same topology and terminal clades as MP trees. *Pestalotiopsis* sensu stricto formed two well supported branches, and the new species *Pestalotiopsis lijiangensis* (CFCC 50738, CFCC 50739) was in a strongly supported independent clade (BP = 100, PP = 1) (FIG. 2).

Discussion

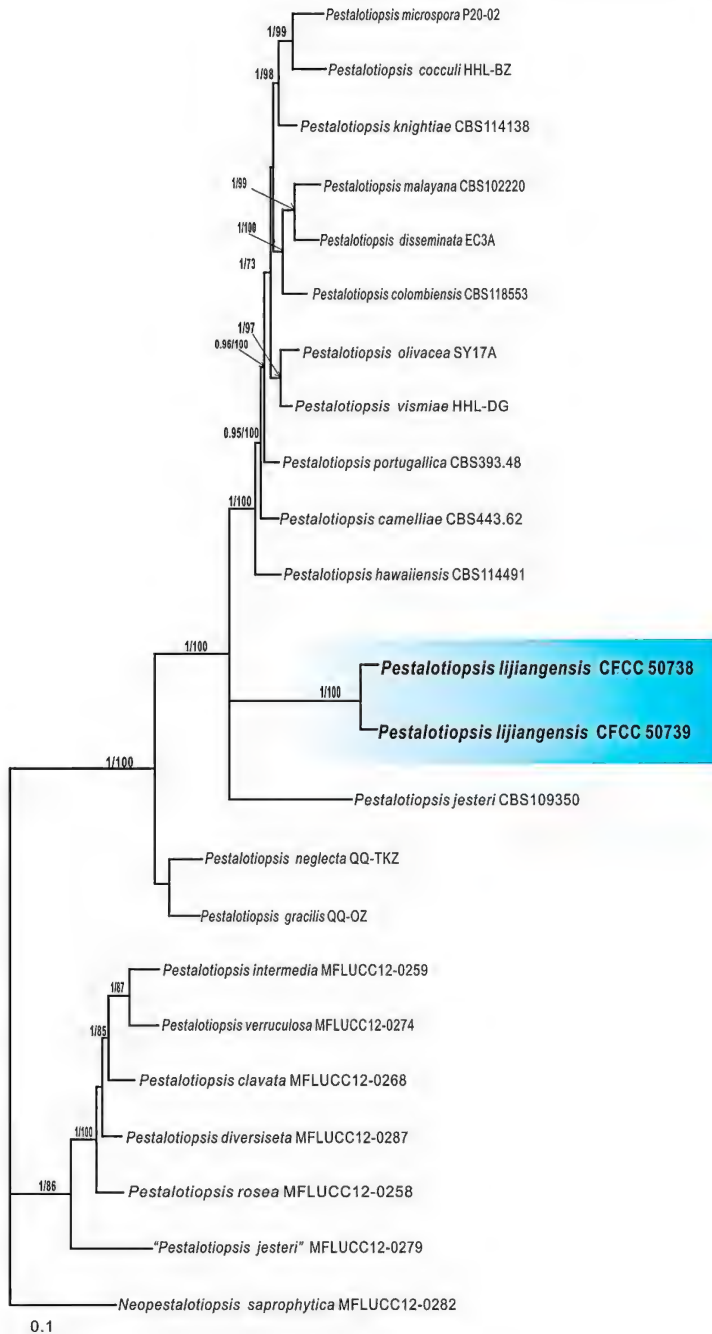
Conidial morphology is the most widely used taxonomic character for species in *Pestalotiopsis*, most species are identified by conidial size (Maharachchikumbura & al. 2011).

As can be seen from TABLE 2, the difference between *P. lijiangensis* and *P. montellica* is mainly the conidial length/width ratio, with the ratio of *P. montellica* almost 1.5 times that of *P. lijiangensis*. Furthermore, the lateral appendages of *P. montellica* are significantly shorter than those of *P. lijiangensis*. Although the conidial length/width ratios of *P. lijiangensis* and *P. jesteri* are similar, the three median cells are remarkably different: in *P. jesteri* the center cell is slightly smaller than the two others, while in *P. jesteri* the center cell is bigger (Strobel & al. 2000). Besides, the conidium of *P. jesteri* has no apical appendages, while *P. lijiangensis* has an unbranched apical appendage. Molecular analyses of the combined datasets cluster *P. lijiangensis* as an outlying species with high branch-length support. Unfortunately, no DNA sequence of *P. montellica* is available, so further research is needed to determine the relationship between *P. lijiangensis* and *P. montellica*.

TABLE 2. Morphological comparison of *Pestalotiopsis lijiangensis* and two similar species

CHARACTERS	SPECIES		
	<i>P. lijiangensis</i> (This paper)	<i>P. montellica</i> (Guba 1961)	<i>P. jesteri</i> (Strobel & al. 2000)
CONIDIUM MORPHOLOGY			
Length (μm)	18.5–27, <i>x</i> = 22.5	22–32, <i>x</i> = 26.5	19–23, <i>x</i> = 19
Width (μm)	6–9, <i>x</i> = 7.5	5–7, <i>x</i> = 5.7	5–7, <i>x</i> = 6
Mean length/width ratio	2.96	4.6	3.2
Median cell shape/color	Doliform/brown	Cylindrical/pale brown	Fusiform/pale brown
Lower median cell (μm)	4.5–6, <i>x</i> = 5.3	6–7.5, <i>x</i> = 6.7	4.8–5.2, <i>x</i> = 5
Center median cell (μm)	4–5.1, <i>x</i> = 4.4	3–5.5, <i>x</i> = 4.6	5.3–5.8, <i>x</i> = 5.5
Upper median cell (μm)	6–7.4, <i>x</i> = 6.3	4–6.5, <i>x</i> = 5.2	4.8–5.1, <i>x</i> = 4.9
APICAL APPENDAGES			
Number	1	1	0
Branching	unbranched	unbranched	—
Length (μm)	2–4, <i>x</i> = 3.4	5–7, <i>x</i> = 6	—
LATERAL APPENDAGES			
Number	(2–)3(–4)	(2–)3(–4)	3
Length (μm)	14–25, <i>x</i> = 18.6	4–12, <i>x</i> = 8	11–28, <i>x</i> = 23
BASAL APPENDAGES			
Number	(0–)1	0(–1)	1
Branching	Unbranched	Unbranched	Unbranched
Length (μm)	2–6, <i>x</i> = 4.8	2–6, <i>x</i> = 4	3–8, <i>x</i> = 4.5

FIG. 2 Phylogenetic tree derived from maximum parsimony analysis of the combined (ITS+TUB+TEF) sequences of *Pestalotiopsis* spp., with *Neopestalotiopsis. saprophytica* as outgroup. Branch support is shown as: Bayesian posterior probabilities >0.95 / bootstrap values >70%.



The two sequenced strains of *P. jesteri* appeared in different clades: CBS 109350 (a type strain from Papua New Guinea) was closely related to *P. lijiangensis*, while MFLUCC 12-0279 (from Yunnan, China) was only distantly related. The Yunnan strain was described with a conidial length/width ratio ≈ 4.1 and sequenced by Maharachchikumbura & al. (2012), who accepted it within *P. jesteri*. The Papua New Guinea strain, originally described by Strobel & al. (2000) and redescribed and sequenced by Maharachchikumbura & al. (2014), has a conidial length/width ratio ≈ 3.2 . The Yunnan strain was excluded from *P. jesteri* by Maharachchikumbura & al. (2014), who included only the original Papua New Guinea material in their circumscription of the species. Further study is needed to identify the misdetermined Yunnan strain MFLUCC 12-0279.

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***Comatricha parvula* sp. nov. and other myxomycetes recorded from Extremadura (Spain) and adjacent areas**

GABRIEL MORENO^{1*}, ÁNGELA LÓPEZ-VILLALBA¹,
AURELIO CASTILLO¹, JUAN RAMÓN GARCÍA²

¹Departamento de Ciencias de la Vida (Botánica), Facultad de Biología,
Universidad de Alcalá, 28805 Alcalá de Henares, Madrid, Spain

²Teodoro de Vera 8, 06920 Azuaga, Badajoz, Spain

* CORRESPONDENCE TO: gabriel.moreno@uah.es

ABSTRACT—Sixty-three myxomycete species from the southwestern Iberian Peninsula are recorded. The most interesting species are commented upon and presented with LM and SEM microphotographs of their distinguishing features. *Comatricha parvula* is described as a new species characterised by its small size and compared with morphologically similar species.

KEY WORDS—*Amoebozoa*, chorology, myxobiota, slime moulds, taxonomy

Introduction

The current work focuses on a Mediterranean area located in the western southwest Iberian Peninsula and considered to be of great biogeographical interest. The region includes the Autonomous Communities of Extremadura, west Castilla-La Mancha and north Andalucía (FIG. 1). Climatically, this area is part of the Mediterranean domain, with a prominent summer dry period and spring and autumn rainfall that can average 650 mm per year. The most studied woodland and shrubland communities are holm oak groves (*Quercus obtusifolia* Lam. [= *Quercus ilex* subsp. *ballota* (Desf.) Samp.]), cork oak groves (*Quercus suber* L.), meadows with holm oaks and cork oaks, Pyrenean oak groves (*Quercus pyrenaica* Willd.), chestnut groves (*Castanea sativa* Mill.),



FIG. 1.. The general study area covered during the research reported herein.

riparian forests (*Salix* spp., *Populus* spp., and *Ulmus* spp.), rockrose scrubs (*Cistus* spp.), pinewoods (*Pinus* spp.), and eucalyptus plantations (*Eucalyptus* spp.) (Arrojo Martín 2006).

The largest number of samples were obtained from “La Campiña Sur” where, as in much of Extremadura and bordering areas, there appear “dehesas” formed mainly by meadows with oaks and cork oaks. In places where human activity is not so obvious, native vegetation remnants appear as holm oak groves along with Mediterranean scrub communities. On the banks of streams and rivers, the dominant vegetation is represented by “adelfares” (*Nerium oleander* L.) and “tamujares” (*Securinega tinctoria* (Loefl.) Rothm.), accompanied by brambles (*Rubus ulmifolius* Schott), reeds (*Juncus* spp.), hawthorn (*Crataegus monogyna* Jacq.), and wild rosebushes (*Rosa* spp.). Prickly pear (*Opuntia ficus-indica* (L.) Mill.) also grows in particular areas.

In spite of the potential number of interesting habitats for myxomycete development, only a few studies have been carried out in this area, not sufficient for properly understanding the myxobiota of these communities. The most important work was carried out in Monfragüe National Park (Cáceres, Extremadura) by Moreno & al. (1990), who tabulated 60 species.

Subsequent studies (Moreno & al. 1991) increased the number of species to 83. In the decade of the 1990's, new sites increased with the studies of García & al. (1996), Illana & al. (1997), and Castillo & al. (2000) and the doctoral theses by Illana (1992) and Castillo (1999). Finally, in the 2000's, Oltra & Lado (2015, 2016) covered myxomycete species from the entire Iberian Peninsula.

Material & methods

The examined material included specimens obtained from field samples (collected mainly by J.R. García) that represented 63 species of myxomycetes. Specimens were deposited in the herbarium of the Life Sciences Department (Botany), University of Alcalá, Madrid, Spain (AH).

Spore measurements (taken using oil immersion) include surface structures such as spines or warts. Light microscopy (LM) was carried out with a Nikon Eclipse 80i microscope equipped with a DS-5M automatic photographic system. Scanning electron micrographs (SEM) were produced by a Zeiss DSM-950 microscope. For ultramicroscopic studies, material was rehydrated in concentrated ammonium hydroxide (28–30%) for 30 min, dehydrated in aqueous ethanol (70%) for 30 min, fixed for 2 h in pure ethylene glycol dimethyl ether (1,2-dimethoxymethane), and finally immersed in pure acetone for at least 2 h. This was followed by critical point drying and sputtering with gold-palladium. This technique allows the use of very little material (i.e., part of a single sporocarp or sometimes no more than a few spores). Terminology used to describe spore ornamentation follows Rammeloo (1974, 1975).

Taxonomy

Arcyria affinis Rostaf., Sluzowkce Monogr.: 276. 1875.

FIG. 2

The sample AH 48694 comprises grouped sporocarps with stalks that reach 0.7 mm long. Capillitium rosy to yellow-creamy, falling easily from the calyculus and expanding over the substrate. Capillitial threads with a variable and patent ornamentation consisting of warts, spines, and cogs, similar to those indicated by Nannenga-Bremekamp (1991). Spores 7–8 μm in diam., globose to subglobose, with the ornamentation typical of *Arcyria* species. Under SEM the inner surface of calyculus is ornamented by a well-developed reticulum. The capillitial tubes with abundant thick half rings and cogs, and spore ornamentation is formed by little baculae and a few groups of more prominent warts.

SPECIMENS EXAMINED: SPAIN, BADAJOZ, Azuaga, Arroyo El Cañaveral, on wood of *Nerium oleander*, 10-I-1993, leg. J.R. García (AH 16468); 24-I-1995, leg. J.R. García (AH 48694 with *Physarum album*); 24-I-1995, leg. J.R. García (AH 48695, with *Comatricha nigra*, *Licea kleistobolus*, and *Physarum album*).

COMMENTS—Our species concept of *Arcyria affinis* follows Nannenga-Bremekamp (1991), who considered *A. similis* (Racib.) Racib. ex Berl. a synonym (see also Lado 2018).

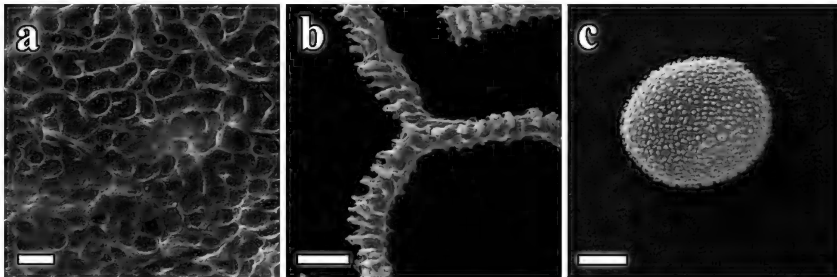


FIG. 2. *Arcyria affinis* (AH 48694). a. Detail of the reticulated inner surface of the calyculus. b. Capillitium. c. Detail of spore ornamentation (SEM). Scale bars: a–c = 2 μm , b = 5 μm .

Arcyria cinerea (Bull.) Pers., Syn. Meth. Fung.: 184. 1801.

FIG. 3

Sporocarps grouped, the capillitium congested, slightly elastic, yellowish white. Spores globose to subglobose, 6–7 μm diam., yellowish grey. Under SEM the inner surface of calyculus is ornamented by faint warts and a fine reticulum. The capillitial tubes abundant and with thick warts; the spores are ornamented by little baculae and a few groups of more prominent warts.

SPECIMENS EXAMINED: SPAIN, BADAJOZ, Azuaga, Arroyo Argallón, on wood of *Nerium oleander*, 30-IX-1989, leg. J.R. García (AH 48649, with *Comatricha anomala*); on wood

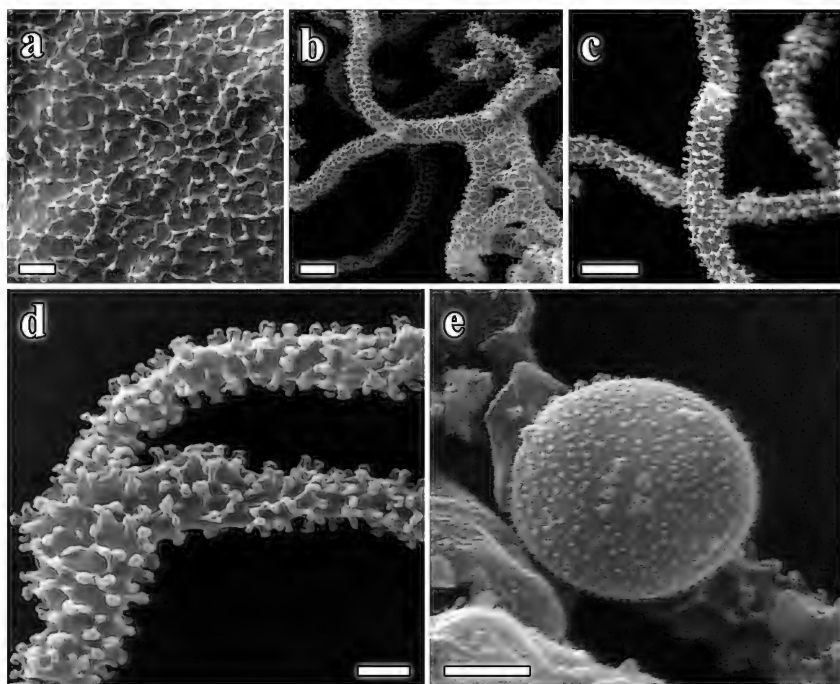


FIG. 3. *Arcyria cinerea* (AH 20688). a. Detail of the calyculus inner surface with warts and a fine reticulum. b-c. Capillitium. d. Detail of capillitium ornamentation (SEM). e. Detail of spore ornamentation (SEM). Scale bars: a, d, e = 2 μ m, b, c = 5 μ m.

of *Nerium oleander*, 7-VI-1991, leg. J.R. García (AH 16372, with *Trichia affinis*); Finca La Sierra, on wood of *Eucalyptus* sp., 15-X-1989, leg. J.R. García (AH 48650); **Granja de Torrehermosa**, Arroyo Quejigo, on wood of *Populus alba*, 9-X-1992, leg. J.R. García (AH 48744, with *Trichia crateriformis*); twigs of *Rubus* sp., 31-X-1992, leg. J.R. García (AH 16325); on wood of *Populus alba*, 24-I-1995, leg. J.R. García (AH 20688, with *Arcyria incarnata*).

COMMENTS—The sample AH 20688 contains two *Arcyria* species, *A. incarnata*, and *A. cinerea*, which can be confused because both produce mature stages that may have yellowish hues. *Arcyria incarnata* is distinguished by its scattered fructifications that are generally globose to subglobose, a capillitium that forms a lax net, and larger (7.1–9.4 μ m diam.) spores (Rammeloo 1981).

Arcyria incarnata (Pers. ex J.F. Gmel.) Pers., *Observ. Mycol.* 1: 58. 1796. FIG. 4

Capillitium elastic, lax, pale yellowish pink. Under SEM the inner surface of the calyculus is ornamented by prominent spines. The tubes of the capillitium have abundant spines, rings, and half rings, and the spore ornamentation is typical of *Arcyria* species.

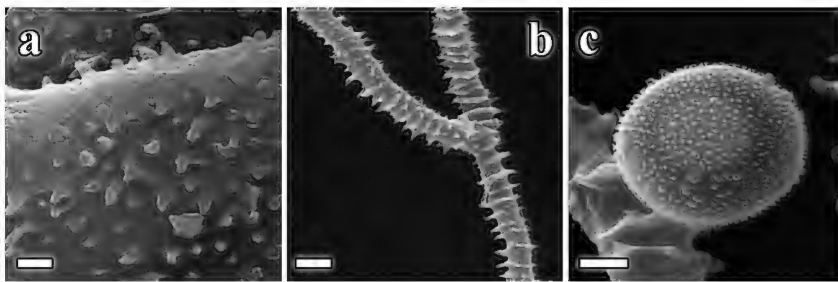


FIG. 4. *Arcyria incarnata* (AH 20688). a. Detail of the calyculus inner surface with spines. b. Capillitium. c. Detail of spore ornamentation (SEM). Scale bars: a-c = 2 μ m, b = 5 μ m.

SPECIMENS EXAMINED: SPAIN, BADAJOZ, Azuaga, Arroyo Argallón, on wood of *Populus alba*, 1-I-1991, leg. J.R. García (AH 48652); on wood of *Rubus* sp., 20-X-1991, leg. J.R. García (AH 48698, with *Comatricha anomala*); Granja de Torrehermosa, Arroyo Quejigo, on wood of *Populus alba*, 24-I-1995, leg. J.R. García (AH 20688, with *Arcyria cinerea*); Peraleda del Zaucejo, Finca Galafate, Puente Arroyo del Madroño, on wood of *Salix fragilis*, 21-X-1994, leg. J.R. García (AH 16267); CÓRDOBA, Fuente Obejuna, Cortijo El Río, debris of *Eucalyptus* sp., 24-XI-1994, leg. J.R. García (AH 48686).

Arcyria minuta Buchet, Mém. Acad. Malagache 6: 42. 1927.

FIG. 5

Sporocarps small, $<3 \times 1.3$ mm, rosy to flesh-coloured. Stalk dark, about 1 mm long. Calyculus <0.7 mm diam. Capillitium firmly attached to the calyculus. Capillitial threads non-elastic, ornamented with rings. Under SEM the capillitial tubes show prominent warts, rings, and half rings, and the spores exhibit the ornamentation typical of *Arcyria* species.

SPECIMENS EXAMINED: SPAIN, BADAJOZ, Azuaga, Arroyo Argallón, on wood of *Nerium oleander*, 11-IX-1991, leg. J.R. García (AH 48697); 2-IV-1991, leg. J.R. García (AH 48651).

COMMENTS—*Arcyria minuta* is characterised by its small size. According to Rammeloo (1981), *Arcyria insignis* Kalchbr. & Cooke can be distinguished

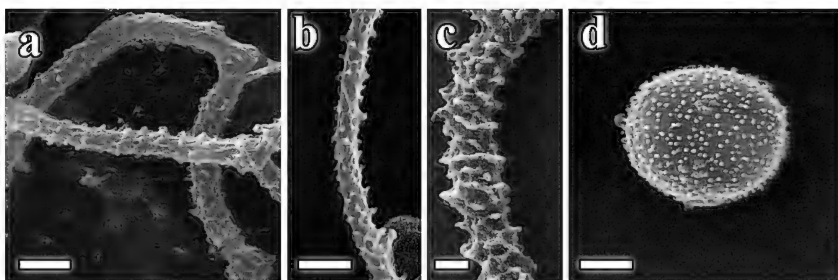


FIG. 5. *Arcyria minuta* (AH48651). a-b. Capillitium. c. Detail of the capillitium ornamentation (SEM). d. Detail of spore ornamentation (SEM). Scale bars: a-b = 5 μ m, c-d = 2 μ m.

from *A. minuta* by its capillitium, which has a less conspicuous ornamentation consisting of spirally arranged warts.

Arcyria obvelata (Oeder) Onsberg, Mycologia 70: 1285. 1979 [“1978”].

SPECIMENS EXAMINED: SPAIN, BADAJOZ, Azuaga, Arroyo Argallón, dried trunk of *Populus* sp., 4-I-1988, leg. J.R. García (AH 48653); on wood of *Nerium oleander*, 11-IX-1991, leg. J.R. García (AH 48654).

Badhamia foliicola Lister, J. Bot. 35: 209. 1897.

SPECIMENS EXAMINED: SPAIN, BADAJOZ, Azuaga, Finca Casas Viejas, leaves of *Quercus obtusifolia* and grasses, 23-VI-1992, leg. J.R. García (AH 48705); Fuentes de León, “Camino del Rincón”, on leaves and twigs of *Quercus obtusifolia*, and grasses, 18-XI-1995, leg. J.R. García (AH 20677); Granja de Torrehermosa, Finca El Río, leaves of *Populus nigra*, 24-VI-1992, leg. J.R. García (AH 16527); CÁCERES, Hoyos, road to Valverde del Fresno, km 4, fronds/blades of *Pteridium aquilinum*, 25-XI-1995, leg. J.R. García (AH 20709); CÓRDOBA, Fuente Obejuna, Finca El Cintado, leaves of *Populus alba*, 27-XI-1995, leg. J.R. García (AH 20713); Cortijo El Río, grasses and debris of *Eucalyptus* sp., 1-XI-1997, leg. J.R. García (AH 46529, with *Physarum cinereum*).

COMMENTS—The sample AH 20713 from Finca El Cintado consists of decarbonated sporocarps, which gives them an iridescent colour. Capillitium badhamioid, formed by very thin filaments. The sample occurred on *Populus alba* leaves, which demonstrates the foliicolous habit of *Badhamia foliicola*.

The sample AH 20677 (on leaves and sticks of *Quercus obtusifolia*) is copious, with the sporocarps forming large colonies. Peridium decarbonated, with violaceous-iridescent colours, with white veins of calcium carbonate coming from the inner capillitium. Capillitium abundant, thin, white, and typically badhamioid. Spores 11–12 µm diam., globose to subglobose, free, pale violet, verrucose.

Finally, the spores of the sample AH 46529 appear in clusters that are easily separated, and some sporocarps have developed a whitish to straw-yellow stalk, such as Lister (1925) indicated.

Badhamia gracilis (T. Macbr.) T. Macbr., Myxomycetes: 35. 1934.

SPECIMENS EXAMINED: SPAIN, BADAJOZ, Azuaga, close to Finca La Paloma, on cladodes of *Opuntia ficus-indica*, 26-X-1990, leg. J.R. García (AH 48660); close to Herrador's Valley, on cladodes of *Opuntia ficus-indica*, 14-IV-1991, leg. J.R. García (AH 48661); close to Finca La Jacoba, on cladodes of *Opuntia ficus-indica*, 10-X-1992, leg. J.R. García (AH 48655); 23-X-1997, leg. J.R. García (AH 48733, with *Physarum spectabile*; AH 48734).

COMMENTS—The sample AH 48661 fruited copiously on cladodes of *Opuntia ficus-indica*. Spores 13–15 µm diam., polyhedric, dark brown, verrucose.

Castillo & al. (1996) studied the type specimen of *Badhamia gracilis*, but our taxonomic concept follows Moreno & Oltra (2010).

Badhamia melanospora Speg., Anales Soc. Ci. Argent. 10: 150. 1880.

SPECIMEN EXAMINED: SPAIN, BADAJOZ, Azuaga, close to Finca La Paloma, on cladodes of *Opuntia ficus-indica*, 26-X-1990, leg. J.R. García (AH 48659).

COMMENTS—The spores of the sample are typical of this species, being 18–20(–22) µm in diam, polyhedric, dark brown, and verrucose. When Castillo & al. (1996) studied the type material of *Badhamia melanospora*, they proposed the illegitimate combination *B. melanospora* var. *gracilis* (Speg.) A. Castillo & al. Moreno & Oltra (2010) later established that *B. melanospora* and *B. gracilis* represent different taxa, and our taxonomic concept follows those authors.

Badhamia panicea (Fr.) Rostaf.,

Jahrb. Nassauischen Vereins Naturk. 27–28: 71. 1873.

SPECIMENS EXAMINED: SPAIN, BADAJOZ, Granja de Torrehermosa, Arroyo Quejigo, bark of *Populus alba*, 26-X-1995, leg. J.R. García (AH 20686); close to Finca Rosa Martínez, 580 m, on wood of *Eucalyptus* sp., 7-XII-1997, leg. J.R. García (AH 48646, with *Comatricha tenerrima*); CÓRDOBA, Fuente Obejuna, Finca Las Canalejas, 540m, on bark of *Nerium oleander*, 12-I-1998, leg. J.R. García (AH 48658).

COMMENTS—The sporocarps of the sample AH 20686 have a badhamioid capillitium that tends to join in the centre of the sporocarp, forming the white pseudocolumella characteristic of *Badhamia panicea*, as indicated by Moreno & Oltra (2010).

Badhamia utricularis (Bull.) Berk., Trans. Linn. Soc. London 21: 153. 1853.

SPECIMENS EXAMINED: SPAIN, BADAJOZ, Azuaga, Finca El Morro, on twigs of *Quercus obtusifolia*, 27-XII-1991, leg. J.R. García (AH 48707); Arroyo Argallón, on wood of *Quercus obtusifolia*, 20-VI-1992, leg. J.R. García (AH 48710); Granja de Torrehermosa, Finca El Revuelo, on wood of *Pinus*, 20-XII-1991, leg. J.R. García (AH 16253); close to Finca El Río, on bark of *Quercus obtusifolia*, 31-XII-1991, leg. J.R. García (AH 48702); Arroyo Quejigo, on wood of *Populus alba*, 22-XII-1994, leg. J.R. García (AH 48684); CÁCERES, Hoyos, at the end of the village, crossroads with the road to Cilleros, on branch of *Quercus suber*, 26-XI-1995, leg. J.R. García (AH 20702).

COMMENTS—The sporocarps of the sample AH 16253 show abundant pseudo-stipes formed by of straw-yellow hypothallus, and clustered spores, which are easily separated under pressure. However, the sporocarps of the sample AH 48707 show a very shrunken or even absent hypothallus, although the spores remain together.

Ceratiomyxa fruticulosa (O.F. Müll.) T. Macbr.,

N. Amer. Slime-Moulds: 18. 1899.

SPECIMEN EXAMINED: SPAIN, BADAJOZ, Azuaga, Arroyo Argallón, 540 m, on bark of *Populus nigra*, 6-XII-1995, leg. J.R. García (AH 20683, with *Trichia varia*).



FIG. 6. *Comatricha anomala* (AH 48701). a. Sporocarp. (AH 48698) b-d. Detail of the spore ornamentation (SEM). Scale bars: a = 200 μ m, b-d = 2 μ m.

***Comatricha anomala* Rammeloo,**

Bull. Jard. Bot. Natl. Belg. 46: 237. 1976.

FIG. 6

SPECIMENS EXAMINED: SPAIN, BADAJOZ, Azuaga, Arroyo Argallón, on wood of *Nerium oleander*, 30-IX-1989, leg. J.R. García (AH 48649, with *Arcyria cinerea*); on wood of *Populus nigra*, 1-IX-1990, leg. J.R. García (AH 48701); on wood of *Populus alba*, 11-X-1991, leg. J.R. García (AH 48699); on debris of *Rubus* sp., 20-X-1991, leg. J.R. García (AH 48698, with *Arcyria incarnata*).

COMMENTS—Sporotheca cylindrical, stalk short in relation to the sporotheca. The spore ornamentation, distinctive in *Comatrucha anomala*, consists of warts that sometimes become attached to form a partial and incomplete reticulum. In Spain, *Comatrucha anomala* fruits mainly on oleander (*Nerium oleander*), poplar (*Populus nigra*), and oak (*Quercus obtusifolia*). Although a globally rare species, *C. anomala* is common in the Mediterranean region. Mainly cited in Europe (<http://www.discoverlife.org>), it is also reported from Mexico, including Baja California (Lizárraga & al. 1997, 2004), Chihuahua (Lizárraga & al. 2005), and Sonora (Lizárraga & al. 2008).

Comatrucha nigra (Pers. ex J.F. Gmel.) J. Schröt., Pilze Schles. 1: 118. 1885.

SPECIMENS EXAMINED: SPAIN, BADAJOZ, Azuaga, Arroyo Argallón, wood of *Nerium oleander*, 1-I-1990, leg. J.R. García (AH 48666); 28-XII-1991, leg. J.R. García (AH 16264, with *Comatrucha parvula*); 22-I-1992, leg. J.R. García (AH 48716); 10-VI-1993, leg. J.R. García (AH 16482, with *Didymium melanospermum* and *Physarum album*); Arroyo El Cañaveral, on wood of *Nerium oleander*, 24-I-1995, leg. J.R. García (AH 48695, with *Arcyria affinis*, *Licea kleistobolus*, and *Physarum album*); 450 m, 26-XII-1996, leg. J.R. García (AH 20676); Granja de Torrehermosa, Arroyo Quejigo, on burnt wood of *Populus alba*, 31-X-1992, leg. J.R. García (AH 48657); on wood of *Populus nigra*, 12-XI-1993, leg. J.R. García (AH 48712); on wood of *Populus alba*, 24-I-1995, leg. J.R. García (AH 20701); 500 m, 27-XI-1995, leg. J.R. García (AH 20706); Trasierra, little meadow, 640 m, on debris of *Pinus pinea*, 15-XI-1997, leg. J.R. García (AH 48644, with *Comatrucha pulchella* and *Craterium leucocephalum*); CÓRDOBA, Fuente Obejuna, Finca Las Canalejas, on bark of *Nerium oleander*, 7-VI-1991, leg. J.R. García (AH 48667); Fuente Obejuna, cortijo del Río, en restos de *Eucalyptus* sp., 24-XI-1994, leg. J.R. García (AH 48687; AH 48688); 27-XI-1995, leg. J.R. García (AH 20711).

Comatrucha parvula G. Moreno, López-Vill., A. Castillo & J.R. García,
sp. nov.

FIG. 7

MYCOBANK MB 825357

Differs from *Comatrucha laxa* by its shorter stalk and its capillitium forming a well-developed external net at the basal half of the sporotheca; from *C. ellae* by its columella with more or less perpendicular main branches; and from *C. variabilis* by its shorter stalk, its capillitium forming a well-developed external net at the basal half of the sporotheca, and its smaller spores.

TYPE: Spain, Badajoz, Azuaga, Arroyo Argallón, on wood of *Populus* sp., 20-VI-1992, leg. J.R. García (Holotype, AH 16428).

ETYMOLOGY: Latin *parvula* (= very small), refers to the size of the sporocarps.

Sporocarps stalked, scattered to gregarious, sometimes growing in large colonies, 1.2–2 mm long. Sporotheca ovoid to slightly prolate, 0.5–0.9 mm long. Stalk as long as the sporotheca or even longer, dark brown with a

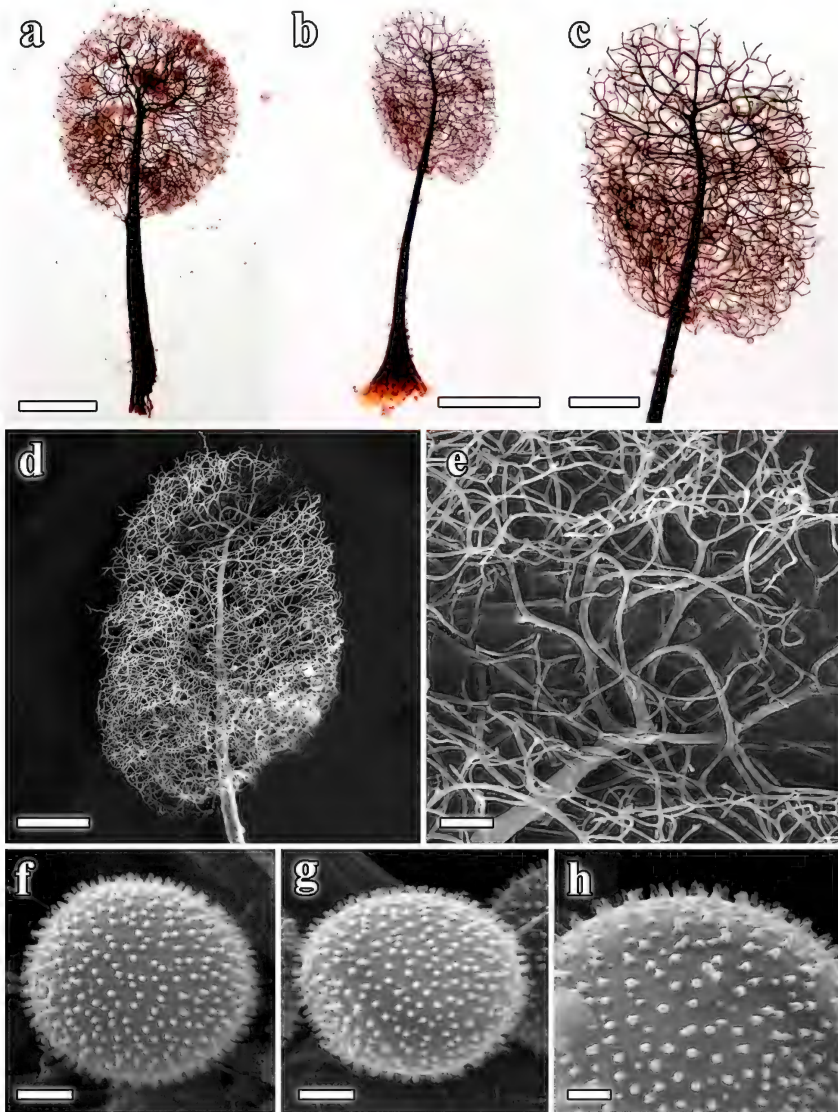


FIG. 7. *Comatricha parvula* (AH 16428 holotype). a-b. Sporocarps. c. Detail of the sporotheca by LM. d. Detail of the sporotheca (SEM). e. Detail of the columella nearly reaching the apex, ending in short branches (SEM). f-g. Spore. h. Detail of spore ornamentation (SEM). Scale bars: a-b = 0.25 mm, c = 0.1 mm, d = 100 μ m, e = 20 μ m, f-g = 2 μ m, h = 1 μ m.

fibrous, reddish-brown base. Columella nearly reaching the apex, ending in 2–5 short branches. Capillitium dense, spreading from the columella with more or less perpendicular main branches, forming a well-developed, external net at the basal half of the sporotheca. Capillitium threads spiny and broadly dichotomous at the ends. Collar present. Spores $9.4\text{--}11.4 \times 9\text{--}11 \mu\text{m}$ diam., av. $10.4 \times 10 \mu\text{m}$, $Q = 1\text{--}1.04$ ($n = 25$), globose to subglobose, violaceous-brown by transmitted light, verrucose. Under SEM we can observe the sporotheca with a dense capillitium formed by filaments with small dispersed spines. The columella ends in several main branches. The spore ornamentation is formed by homogenously distributed baculae.

HABITAT & DISTRIBUTION: Occurring on the wood and bark of such species as *Nerium oleander*, *Pinus* spp., *Populus alba*, and *Populus nigra* and currently known only from Badajoz (Extremadura) and Córdoba (Andalucía), Spain.

ADDITIONAL SPECIMENS EXAMINED: **SPAIN, BADAJOZ, Azuaga**, Arroyo Argallón, on wood of *Nerium oleander*, 28-XII-1991, leg. J.R. García (AH 16264, with *Comatriza nigra*); on wood of *Nerium oleander*, 10-VI-1993, leg. J.R. García (AH 16279); **Granja de Torrehermosa**, Arroyo Quejigo, on wood of *Populus alba*, 31-X-1992, leg. J.R. García (AH 16432); on wood of *Populus nigra*, 12-XI-1993, leg. J.R. García (AH 16366); on burnt wood of *Populus alba*, 4-XI-1994, leg. J.R. García (AH 18434); 19-XI-1994, leg. J.R. García (AH 18437); Finca El Revuelo de Hermenegildo, on wood of *Pinus* sp., 12-XII-1992, leg. J.R. García (AH 15505); **CÓRDOBA, Fuente Obejuna**, Cortijo El Río, on bark of *Populus alba*, 27-XI-1995, leg. J.R. García (AH 20714).

COMMENTS—*Comatriza parvula* is characterised by its small size, a capillitium that forms a net in the basal half of the sporotheca but is more open towards the apex, and a columella ramifying at various points at the apex.

A morphologically similar species is *Comatriza laxa* Rostaf., which has a longer (c. 1 mm) stalk and a capillitium that is totally lax and hence does not form an external net. *Comatriza ellae* Härk. is also a small species, but its capillitium is more lax than in *C. parvula* and lacks branches at the apex of the columella. *Comatriza variabilis* R.K. Chopra & T.N. Lakh. has longer stalks (twice the length of the sporotheca), its capillitium lacks both a well-developed net at the sporothecal base and branches at the columellar apex, and its spores are larger ($10\text{--}12.5 \mu\text{m}$ diam.).

Comatriza microcarpa (Meyl.) Kowalski, another similar species, has spores $11\text{--}13 \mu\text{m}$ diam. and a sparse rigid capillitium that often comprises only 5 or 6 major branches, which further divide one or two times to taper towards the free extremities without anastomosing (Kowalski 1975). Kowalski compared *C. microcarpa* with *Paradiacheopsis rigida* (Brândză) Nann.-Bremek., and indicated that it probably represents the most closely related taxon. Moreno

& al. (2018) carried out a study of *P. rigida* and proposed a lectotype, which is conserved in BM. *Comatricha microcarpa* was considered a nomen ambiguum by Castillo & al. (1997).

Comatricha pulchella (C. Bab.) Rostaf., Sluzowkce Monogr. Suppl.: 27. 1876.

SPECIMEN EXAMINED: SPAIN, BADAJOZ, Trasierra, little meadow, 640 m, on debris of *Pinus pinea*, 15-XI-1997, leg. J.R. García (AH 48644, with *Comatricha nigra* and *Craterium leucocephalum*).

Comatricha tenerrima (M.A. Curtis) G. Lister,

Guide Brit. Mycetoza, ed. 4: 39. 1919.

SPECIMENS EXAMINED: SPAIN, BADAJOZ, Azuaga, Arroyo Argallón, on debris of *Heracleum mantegazzianum* and *Epilobium hirsutum*, 1-XI-1997, leg. J.R. García (AH 48753, AH 48754); Granja de Torrehermosa, close to Finca Rosa Martínez, 580 m, on wood of *Eucalyptus* sp., 7-XII-1997, leg. J.R. García (AH 48646, with *Badhamia panicea*); Maguilla, Finca Las Ventas, on plant debris, 1-XI-1995, leg. J.R. García (AH 20703).

Craterium leucocephalum (Pers. ex J.F. Gmel.) Ditmar,

Deutschl. Fl. Pilze 1(1): 21. 1813.

SPECIMENS EXAMINED: SPAIN, BADAJOZ, Azuaga, Arroyo Argallón, 540 m, on wood of *Nerium oleander* and twigs of *Rubus* sp., 19-X-1997, leg. J.R. García (AH 46530, with *Stemonitis fusca*); Finca Casas Viejas, 380 m, on leaves of *Quercus obtusifolia*, 28-XII-1995, leg. J.R. García (AH 20684, with *Didymium clavus*); Trasierra, little meadow, 640 m, on debris of *Pinus pinea*, 15-XI-1997, leg. J.R. García (AH 48644, with *Comatricha nigra* and *C. pulchella*).

Craterium minutum (Leers) Fr., Syst. Mycol. 3: 151. 1829.

SPECIMENS EXAMINED: SPAIN, BADAJOZ, Azuaga, Arroyo Argallón, on twigs of *Rubus* sp., 1-XII-1990, leg. J.R. García (AH 48668); 11-I-1991, leg. J.R. García (AH 48678); on fallen branch of *Quercus obtusifolia*, 1-II-1991, leg. J.R. García (AH 48680); CÓRDOBA, Fuente Obejuna, Finca Las Canalejas, on twigs of *Rubus* sp., 11-I-1991, leg. J.R. García (AH 48677).

Cribraria cancellata (Batsch) Nann.-Bremek.,

Nederlandse Myxomyceten: 92. 1975 [“1974”].

SPECIMEN EXAMINED: SPAIN, BADAJOZ, Azuaga, Arroyo Argallón, on dry twigs of *Nerium oleander*, 10-XII-1989, leg. J.R. García (AH 48679).

Cribraria violacea Rex, Proc. Acad. Nat. Sci. Philadelphia 43: 393. 1891.

SPECIMENS EXAMINED: SPAIN, BADAJOZ, Azuaga, Arroyo Argallón, on wood of *Nerium oleander*, 11-I-1991, leg. J.R. García (AH 48690, with *Hemitrichia clavata*); 27-VII-1991, leg. J.R. García (AH 48704).

Diachea leucopodia (Bull.) Rostaf., Sluzowkce Monogr.: 190. 1874.

SPECIMEN EXAMINED: SPAIN, CÓRDOBA, Fuente Obejuna, Cortijo El Río, grasses and debris of *Eucalyptus* sp., 1-XI-1997, leg. J.R. García (AH 46527).

Dictydiaethalium plumbeum (Schumach.) Rostaf., Monogr. Mycetoza: 157. 1984.

SPECIMEN EXAMINED: SPAIN, BADAJOZ, Azuaga, Cortijo La Herejía, 550 m, on bark of *Populus nigra*, 26-I-1998, leg. J.R. García (AH 46528).

COMMENTS—The sample shows the typical remnants of the silvery hypothallus covering the aethalium and the substrate. Spores 8–10 µm diam., globose to subglobose, hyaline-yellow in transmitted light, free, spiny.

Didymium bahiense Gottsb., Nova Hedwigia 15: 365. 1968.

SPECIMENS EXAMINED: SPAIN, BADAJOZ, Azuaga, Finca La Jacoba, on leaves of *Quercus obtusifolia*, 6-III-1992, leg. J.R. García (AH 16338, with *Didymium clavus*, *D. megalosporum*, and *D. squamulosum*); close to Finca La Jacoba, on leaves and twigs of *Quercus obtusifolia*, 6-III-1992, leg. J.R. García (AH 48715, with *Didymium megalosporum*); Granja de Torrehermosa, Granja-Peraleda road, km 10, Zújar rives, on leaves of *Populus alba*, 15-X-1993, leg. J.R. García (AH 48669).

COMMENTS—The sample AH 48715 occurs along with *Didymium megalosporum*. *Didymium bahiense* has a discoid, whitish pseudocolumella, hyaline capillitial threads radiating from the pseudocolumella and becoming attached to the peridium. In contrast, *D. megalosporum* shows a flat, discoid, yellow-orange pseudocolumella, a larger sporotheca (0.7–0.9 mm diam.), and a paler stalk ranging in color from orange-brown to reddish-brown.

Didymium clavus (Alb. & Schwein.) Rabenh., Deutschl. Krypt.-Fl. 1: 280. 1844.

SPECIMENS EXAMINED: SPAIN, BADAJOZ, Azuaga, Finca La Jacoba, on leaves of *Quercus obtusifolia*, 6-III-1992, leg. J.R. García (AH 16338, with *Didymium bahiense*, *D. megalosporum*, and *D. squamulosum*); Finca Casas Viejas, 380 m, on leaves of *Quercus obtusifolia*, 26-XII-1995, leg. J.R. García (AH 20678); 28-XII-1995, leg. J.R. García (AH 20684, with *Craterium leucocephalum*).

Didymium difforme (Pers.) Gray, Nat. Arr. Brit. Pl. 1: 571. 1821.

SPECIMENS EXAMINED: SPAIN, BADAJOZ, Azuaga, Arroyo Argallón, on grasses, 1-XII-1990, leg. J.R. García (AH 48673); Granja de Torrehermosa, Arroyo Quejigo, on leaves of *Populus* sp. and *Juncus* sp., 22-XII-1994, leg. J.R. García (AH 48685, with *Didymium squamulosum*); CÓRDOBA, Fuente Obejuna, Cortijo El Río, on grasses (remains of a nest) under *Eucalyptus* sp., 1-XI-1997, leg. J.R. García (AH 48741).

Didymium laxifilum G. Lister & J. Ross, Essex Naturalist 27: 264. 1945. FIG. 8

SPECIMEN EXAMINED: SPAIN, BADAJOZ, Peraleda del Zaucejo, Fuente del Juncal, 600 m, on debris of *Eucalyptus* sp., 31-XII-1995, leg. J.R. García (AH 20685).

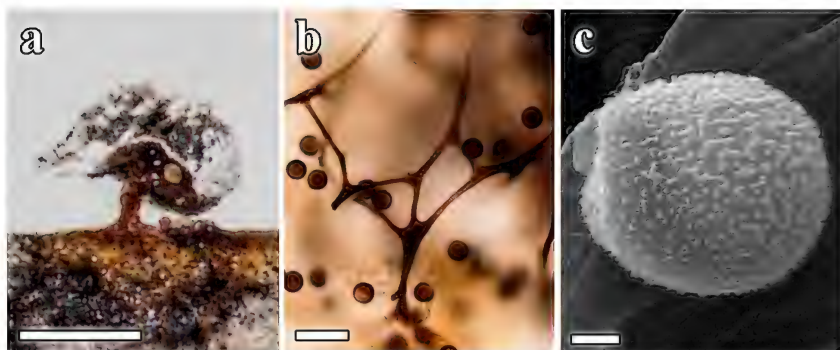


FIG. 8. *Didymium laxifilum* (AH 20685). a. Sporocarp. b. Capillitium. c. Detail of spore ornamentation (SEM). Scale bars: a = 1 mm, b = 25 μ m, c = 2 μ m.

COMMENTS—The differences between *D. laxifilum* and *D. rubeopus* are described below, as part of the description of the latter species. Under SEM, the spore ornamentation comprises warts that join to form short crests.

Didymium megalosporum Berk. & M.A. Curtis, Grevillea 2: 53. 1873.

The sample AH 20720 is copious and well developed. The morphological variability of the sporotheca is clearly shown. The sporotheca is irregular, varying from reniform to discoid and even from flat to lenticular; it is also umbilicate at the union with the stalk. Peridium slightly yellow, covered by white calcium carbonate crystals. Stalk longer than the sporotheca, brown to reddish-brown. Pseudocolumella well developed, flat, yellow to orange-yellow, rough. Capillitium emerging from the pseudocolumella and getting attached to the peridium. Spores 10–11 μ m diam., globose to subglobose, violaceous-brown, verrucose.

SPECIMENS EXAMINED: SPAIN, BADAJOZ, Azuaga, close to Herrador valley, on twigs and leaves of *Quercus obtusifolia*, 19-I-1992, leg. J.R. García (AH 48656); close to Finca La Jacoba, on leaves and twigs of *Quercus obtusifolia*, 6-III-1992, leg. J.R. García (AH 48715, with *Didymium bahiense*); Finca La Jacoba, on leaves of *Quercus obtusifolia*, 6-III-1992, leg. J.R. García (AH 16338, with *Didymium bahiense*, *D. clavus*, and *D. squamulosum*); CÁCERES, Hoyos, at the end of the village, crossroads with the road to Cilleros, on leaves of *Quercus suber*, 26-XI-1995, leg. J.R. García (AH 20720); CÓRDOBA, Fuente Obejuna, Cortijo El Río, on grasses and debris of *Eucalyptus* sp., 1-XI-1997, leg. J.R. García (AH 46531, with *Didymium nigripes* and *Physarum pusillum*); Finca El Cintado, on debris of *Eucalyptus* sp., 27-XI-1995, leg. J.R. García (AH 20712, with *Didymium nigripes* and *D. minus*).

Didymium melanospermum (Pers.) T. Macbr., N. Amer. Slime-Moulds: 88. 1899.

SPECIMENS EXAMINED: SPAIN, BADAJOZ, Azuaga, Arroyo Argallón, on leaves of *Populus nigra*, 10-VI-1993, leg. J.R. García (AH 16446); on wood of *Nerium oleander*, 10-VI-1993, leg. J.R. García (AH 16482, with *Comatricha nigra* and *Physarum leucophaeum*); Trasierra, little meadow, 640 m, on debris of *Cistus* sp., under *Pinus pinea*, 15-XI-1997, leg. J.R. García (AH 48645).

Didymium minus (Lister) Morgan, J. Cincinnati Soc. Nat. Hist. 16: 145. 1894.

SPECIMENS EXAMINED: SPAIN, BADAJOZ, Granja de Torrehermosa, Arroyo Quejigo, on leaves of *Populus nigra*, 12-XI-1993, leg. J.R. García (AH 48714); CIUDAD REAL, Parque Nacional de Cabañeros, leaves, 18-X-2001, leg. G. Moreno (AH 48749).

Didymium nigripes (Link) Fr., Syst. Mycol. 3: 119. 1829.

SPECIMENS EXAMINED: SPAIN, BADAJOZ, Pallares, road to Ventas del Culebrín, km. 2, on debris of *Populus alba*, *Ulmus* sp., and *Juncus* sp., 27-XI-1995, leg. J.R. García (AH 20719); Azuaga, Arroyo Argallón, on non-determined leaves and twigs, leg. J.R. García (AH 16259, with *Physarum album*, and *Physarum leucophaeum*); on debris of grasses, X-1990, leg. J.R. García (AH 16746); on debris of *Rubus* sp. and *Nerium oleander*, 19-X-1997, leg. J.R. García (AH 48751); on debris of *Heracleum mantegazzianum* and *Mentha suaveolens*, 1-XI-1997, leg. J.R. García (AH 46471, with *Lamproderma scintillans*); Granja de Torrehermosa, Arroyo Quejigo, on leaves of *Populus nigra*, 12-XI-1993, leg. J.R. García (AH 48711); on leaves of *Populus alba*, 24-XI-1995, leg. J.R. García (AH 20715); Granja-Peraleda road, km 10, río Zújar, on leaves of *Populus alba*, 15-X-1992, leg. J.R. García (AH 16307); Finca El Río, Zújar river, on debris of grasses and *Juncus* sp. under *Quercus obtusifolia*, 24-VI-1992, leg. J.R. García (AH 16175, with *Physarum leucophaeum*); Maguilla, Finca Las Ventas, on debris of grasses and mosses, 1-XI-1995, leg. J.R. García (AH 20698); CÓRDOBA, Fuente Obejuna, Cortijo El Río, on grasses and debris of *Eucalyptus* sp., 1-XI-1997, leg. J.R. García (AH 46531, with *Didymium megalosporum* and *Physarum pusillum*); Finca El Cintado, on debris of *Eucalyptus* sp., 27-XI-1995, leg. J.R. García (AH 20712, with *Didymium megalosporum*).

Didymium rubeopus G. Moreno, A. Castillo & Illana,

Congr. Int. Sist. Ecol. Myxomycetes: 57. 1996.

FIG. 9

SPECIMEN EXAMINED: SPAIN, CÁCERES, Hoyos, at the end of the village, crossroads with the road to Cilleros, on leaves of *Quercus suber*, 26-XI-1995, leg. J.R. García (AH 20690).

COMMENTS—*Didymium rubeopus* is closely related macroscopically to other foliicolous species such as *D. laxifilum*. However, *D. rubeopus* shows a capillitium formed by narrower (2–4 µm diam.) threads, compared to 5–8 µm diam. of *D. laxifilum*, its spores are smaller, 9–11 µm diam. (10–13 µm diam. for *D. laxifilum*), and its spores are ornamented by tall pila distributed evenly over the surface and sometimes attached to each other. In contrast, the spores of *D. laxifilum* have short crests (Moreno & al. 1997).

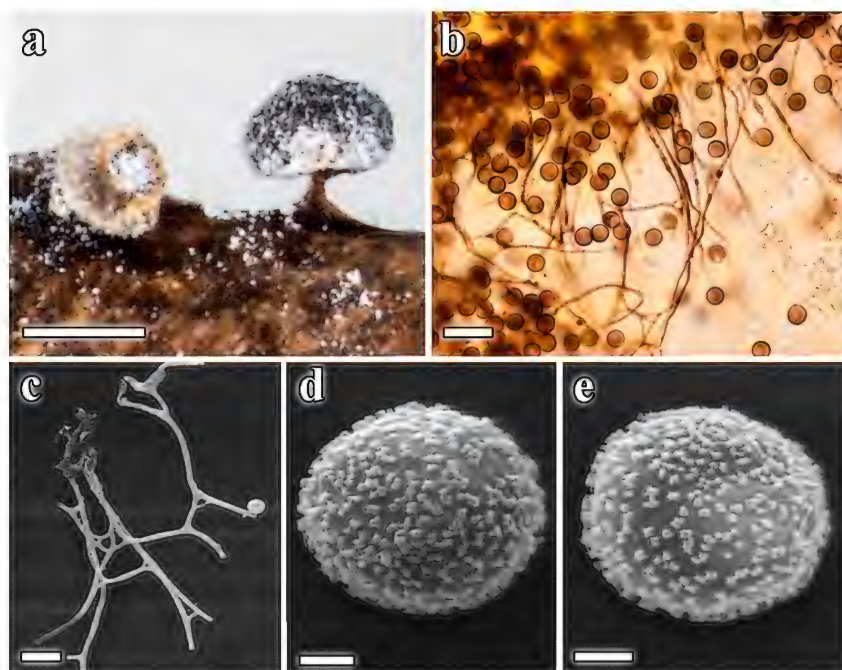


FIG. 9. *Didymium rubeopus* (AH 20690). a. Sporocarps. b. Capillitium. c. Detail of ramifications of the capillitium (SEM). d-e. Detail of spore ornamentation (SEM). Scale bars: a = 1 mm, b = 25 μ m, c = 20 μ m, d-e = 2 μ m.

Didymium squamulosum (Alb. & Schwein.) Fr., Symb. Gasteromyc.: 19. 1818.

SPECIMENS EXAMINED: SPAIN, BADAJOZ, Pallares, road to Ventas del Culebrín, km 2, on debris of *Populus alba*, *Ulmus* sp., and grasses, 3-XII-1995, leg. J.R. García (AH 20693); Azuaga, Arroyo Argallón, on leaves and twigs of *Rubus* sp., 20-I-1990, leg. J.R. García (AH 48674); on leaves of *Nerium oleander*, 12-XII-1991, leg. J.R. García (AH 16258); Arroyo El Cañaveral, on leaves of *Nerium oleander*, 10-I-1993, leg. J.R. García (AH 16174); on leaves of *Quercus obtusifolia*, 10-I-1993, leg. J.R. García (AH 48748); 450 m, on debris of *Nerium oleander*, 26-XII-1996, leg. J.R. García (AH 20682); Cortijo La Herejía, 550 m, on leaves of *Ulmus minor*, 26-I-1998, leg. J.R. García (AH 48739, AH 48740); Finca La Jacoba, on leaves of *Quercus obtusifolia*, 6-III-1992, leg. J.R. García (AH 16338, with *Didymium bahiense*, *D. clavus*, and *D. megalosporum*); Granja de Torrehermosa, Arroyo Quejigo, on debris of *Populus nigra*, 12-XI-1993, leg. J.R. García (AH 48706, with *Physarum leucophaeum*; AH 48708); on leaves of *Populus* sp. and *Juncus* sp., 22-XII-1994, leg. J.R. García (AH 48685, with *Didymium difforme*); on debris of *Juncus* sp. and grasses, 24-I-1995, leg. J.R. García (AH 20718); Peraleda del Zaucejo, Las Hoyas mountain pass, on twigs and leaves of *Quercus suber*, 12-XII-1989, leg. J.R. García (AH 48675); CÁCERES, Hoyos, at the end of the village, crossroads with the road to Cilleros, on leaves and twigs of *Quercus suber*, 26-XI-1995, leg. J.R. García (AH 20708);

AH 20716, with *Physarum album* and *P. bivalve*); CÓRDOBA, Fuente Obejuna, Finca Las Canalejas, on leaves of *Nerium oleander*, 29-XI-1991, leg. J.R. García (AH 48676); Cortijo El Río, on debris of *Eucalyptus* sp., 1-XI-1997, leg. J.R. García (AH 46467; AH 48742).

COMMENTS—Sample AH 48740 illustrates the great morphological variability of *Didymium squamulosum*, consisting of both stalked and sessile sporocarps. Peridium greyish to white. Pseudocolumella patent, flat to subglobose, white to slightly yellow. Stalk with calcium carbonate that varies in color from white to straw-yellow. Capillitium and spores also quite variable. All these characteristics lead us to regard this morphospecies as a species complex.

Didymium vaccinum (Durieu & Mont.) Buchet,

Bull. Soc. Mycol. France 36: 110. 1920.

All three samples show a very pronounced and convex pseudocolumella, capillitial threads emerging perpendicularly to the pseudocolumella, and spores 8–10 µm diam., globose to subglobose, with very conspicuous warts.

SPECIMENS EXAMINED: SPAIN, BADAJOZ, Azuaga, Finca La Jacoba, on cladodes of *Opuntia ficus-indica*, 2-I-1992, leg. J.R. García (AH 48681); 6-III-1992, leg. J.R. García (AH 48700); 16-I-1994, leg. J.R. García (AH 48663).

COMMENTS—*Didymium vaccinum* is usually associated with other succulenticolous species such as *Badhamia gracilis* and *Physarum spectabile*. The spore ornamentation has been studied by Moreno & al. (2001).

Hemitrichia clavata (Pers.) Rostaf.,

Jahrb. Nassauischen Vereins Naturk. 27–28: 75. 1873.

SPECIMENS EXAMINED: SPAIN, BADAJOZ, Azuaga, Arroyo Argallón, on wood of *Nerium oleander*, 1-XI-1991, leg. J.R. García (AH 48690, with *Cribraria violacea*); CÓRDOBA, Fuente Obejuna, Finca Las Canalejas, on wood of *Populus* sp., 9-XI-1991, leg. J.R. García (AH 48691; AH 48692).

Hemitrichia succulenticola G. Moreno, A. Castillo, López-Vill. & A. Sánchez, Bol. Soc. Micol. Madrid 41: 32. 2017.

SPECIMEN EXAMINED: SPAIN, BADAJOZ, Azuaga, close to Finca La Jacoba, on cladodes of *Opuntia ficus-indica*, 6-III-1992, leg. J.R. García (AH 48703).

COMMENTS—This species was described recently by Moreno & al. (2017a). It normally occurs on fallen cladodes of *Opuntia ficus-indica*. *Hemitrichia succulenticola* is characterised by its capillitium formed by threads with spiny spirals, spiny swellings, and a few free ends represented as short tips. Spores 11–13 µm diam.

Lamproderma scintillans (Berk. & Broome) Morgan,

J. Cincinnati Soc. Nat. Hist. 16: 131. 1894.

SPECIMENS EXAMINED: SPAIN, BADAJOZ, Azuaga, Arroyo Argallón, on debris of *Heracleum mantegazzianum* and *Mentha suaveolens*, 1-XI-1997, leg. J.R. García (AH 46471, with *Didymium nigripes*); Granja de Torrehermosa, Granja-Peraleda road, km 10, Zújar river, on leaves of *Salix fragilis*, 15-X-1992, leg. J.R. García (AH 48670); CÓRDOBA, Fuente Obejuna, Cortijo El Río, on grasses and debris of *Eucalyptus* sp., 1-XI-1997, leg. J.R. García (AH 46470).

Leocarpus fragilis (Dicks.) Rostaf., Sluzowkce Monogr.: 132. 1874.

SPECIMENS EXAMINED: SPAIN, BADAJOZ, Azuaga, close to Finca La Jacoba, on leaves of *Quercus obtusifolia* and on cladodes of *Opuntia ficus-indica*, 16-I-1994, leg. J.R. García (AH 48664, with *Physarum spectabile*); Mérida, Cornalvo Roman water reservoir, on twigs of *Quercus suber* and leaves of *Cistus* sp., 20-XI-1993, leg. J.R. García (AH 48665); CÁCERES, Almaraz, on rocks and leaves, 9-XI-1991, leg. G. Moreno (AH 46472); Hoyos, at the end of the village, crossroads with the road to Cilleros, on leaves of *Quercus suber*, 26-XI-1995, leg. J.R. García (AH 20695); Navalmoral de la Mata, La Bazagona pine forest, on plant debris, 6-XII-2000, leg. G. Moreno (AH 46473).

Licea kleistobolus G.W. Martin, Mycologia 34: 702. 1942.

SPECIMEN EXAMINED: SPAIN, BADAJOZ, Azuaga, Arroyo El Cañaveral, on wood of *Nerium oleander*, 24-I-1995, leg. J.R. García (AH 48695, with *Arcyria affinis*, *Comatricha nigra*, and *Physarum album*).

Lycogala flavofuscum (Ehrenb.) Rostaf.,

Jahrb. Nassauischen Vereins Naturk. 27–28: 68. 1873.

SPECIMENS EXAMINED: SPAIN, BADAJOZ, Azuaga, Arroyo Argallón, 540 m, on wood of *Populus nigra*, 29-VI-1997, leg. J.R. García (AH 48746); Granja de Torrehermosa, Arroyo Quejigo, on wood of *Populus* sp., 12-X-1992, leg. J.R. García (AH 48683); CÁCERES, Cáceres, in city center, on leaf litter of *Quercus* sp., 18-VII-2015, leg. Soc. Micol. Madrid (AH 46526).

Mucilago crustacea F.H. Wigg., Prim. Fl. Holsat.:112. 1780.

SPECIMEN EXAMINED: SPAIN, CÁCERES, Almaraz, on dried grasses (*Poaceae*), 9-XI-1999, leg. G. Moreno (AH 48747).

Perichaena depressa Lib., Pl. Crypt. Arduenna: 378. 1837.

SPECIMEN EXAMINED: SPAIN, BADAJOZ, Azuaga, Cortijo La Herejía, on bark of *Populus nigra*, 26-I-1998, leg. J.R. García (AH 46469).

Physarum album (Bull.) Chevall., Fl. Gén. Env. Paris 1: 336. 1826.

FIG. 11a

SPECIMENS EXAMINED: SPAIN, BADAJOZ, Pallares, road to Ventas del Culebrín, km 2, on debris of *Populus alba*, 3-XII-1995, leg. J.R. García AH 16424, with *Physarum*

cinereum); **Azuaga**, Arroyo Argallón, on non-determined leaves and twigs, 29-I-1994, leg. J.R. García (AH 16259, with *Didymium nigripes* and *Physarum leucophaeum*); on wood of *Nerium oleander*, 10-VI-1993, leg. J.R. García (AH 16482, with *Comatricha nigra* and *Didymium melanospermum*); Arroyo El Cañaveral, on wood of *Nerium oleander*, 24-I-1995, leg. J.R. García (AH 48694, with *Arcyria affinis*; AH 48695, with *Arcyria affinis*, *Comatricha nigra*, and *Licea kleistobolus*); **Granja de Torrehermosa**, Finca El Revuelo, on wood of *Pinus* sp., 12-XII-1992, leg. J.R. García (AH 16252); **Mérida**, Cornalvo Roman water reservoir, on wood of *Quercus obtusifolia*, 20-XI-1993, leg. J.R. García (AH 48672); **CÁCERES**, Hoyos, road to Valverde del Fresno, km 4, on wood and leaves of *Castanea sativa*, 25-XI-1995, leg. J.R. García (AH 20704); on wood and leaves of *Castanea sativa* and leaves of *Quercus pyrenaica*, 25-XI-1995, leg. J.R. García (AH 20710); at the end of the village, crossroads with the road to Cilleros, on leaves of *Quercus suber*, 26-XI-1995, leg. J.R. García (AH 20716, with *Didymium squamulosum* and *Physarum bivalve*); **CÓRDOBA**, **Fuente Obejuna**, Cortijo El Río, on debris of *Eucalyptus* sp., 1-XI-1997, leg. J.R. García (AH 46467).

Physarum bethelii T. Macbr. ex G. Lister, Monogr. Mycetoza, ed. 2: 57. 1911.

SPECIMEN EXAMINED: SPAIN, BADAJOZ, Azuaga, Cortijo La Herejía, 550 m, on bark of *Populus nigra*, 29-I-1998, leg. J.R. García (AH 46466).

Physarum bivalve Pers., Ann. Bot. (Usteri) 15: 5. 1795.

SPECIMEN EXAMINED: SPAIN, CÁCERES, Hoyos, at the end of the village, crossroads with the road to Cilleros, on leaves of *Quercus suber*, 26-XI-1995, leg. J.R. García (AH 20716, with *Didymium squamulosum* and *Physarum album*).

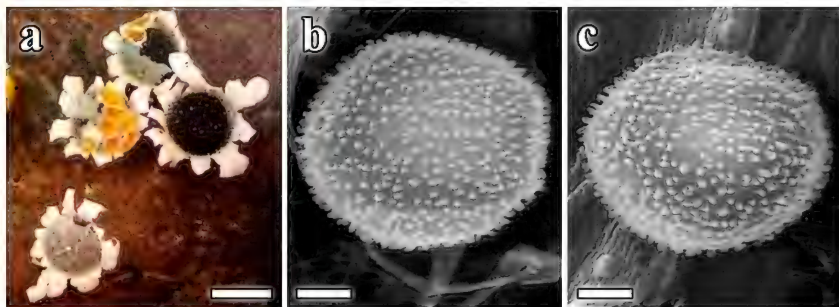


FIG. 10. *Physarum brunneolum* (AH 20680). a. Sporocarps. b-c. Detail of spore ornamentation (SEM). Scale bars: a = 1 mm, b-c = 2 μ m.

Physarum brunneolum (W. Phillips) Masee,

Monogr. Myxogastr.: 280. 1892.

FIG. 10

SPECIMENS EXAMINED: SPAIN, BADAJOZ, Azuaga, Finca Casas Viejas, 380 m, on leaves of *Quercus obtusifolia*, 26-XII-1995, leg. J.R. García (AH 20680).

COMMENTS—The mature peridium of these sporocarps breaks apart in a tessellated fashion, in contrast with the dehiscence observed by Poulain

& al. (2011). Under SEM the spore ornamentation is formed by baculae homogeneously scattered over the surface.

Physarum cinereum (Batsch) Pers., Neues Mag. Bot. 1: 89. 1794.

SPECIMENS EXAMINED: SPAIN, BADAJOZ, Pallares, road to Ventas del Culebrín, km 2, on remains of *Populus alba*, 3-XII-1995, leg. J.R. García (AH 16424, with *Physarum album*); 540 m, on branch of *Rubus* sp., 3-XII-1995, leg. J.R. García (AH 20707); Azuaga, finca «Casas Viejas», 380 m, on leaves of *Quercus obtusifolia*, 26-XII-1995, leg. J.R. García (AH 20679); Granja de Torrehermosa, finca «El Río», on leaves of *Populus alba*, 7-VII-1992, leg. J.R. García (AH 16332); arroyo Quejigo, on remains of herbaceous plants, 24-XI-1995, leg. J.R. García (AH 20692); CÁCERES, Hoyos, road to Valverde del Fresno, km 4, on leaves of *Castanea sativa* and *Quercus pyrenaica*, 25-XI-1995, leg. J.R. García (AH 20699); road junction of Cilleros, on leaves of *Quercus suber*, 26-XI-1995, leg. J.R. García (AH 20717); CÓRDOBA, Fuente Obejuna, cortijo del Río, on remains of herbaceous plants and wood of *Eucalyptus* sp., 1-XI-1997, leg. J.R. García (AH 46529).

Physarum clavisporem G. Moreno, A. Sánchez,

A. Castillo & Illana, Bol. Soc. Micol. Madrid 33: 143. 2009.

SPECIMEN EXAMINED: SPAIN, BADAJOZ, Granja de Torrehermosa, close to the river, on stems of *Foeniculum vulgare*, 27-XII-1993, leg. J.R. García (AH 48662).

COMMENTS—The sample is sparse, consisting of only one sporocarp and a semi-permanent slide. *Physarum clavisporem*, described by Moreno & al. (2009), was collected recently in Chile by Lado & al. (2013).

Physarum hongkongense Chao H. Chung, Slime Moulds Hong Kong: 19. 1997.

SPECIMEN EXAMINED: SPAIN, BADAJOZ, Azuaga, Cortijo La Herejía, on leaves of *Populus nigra*, 26-I-1998, leg. J.R. García (AH 46468).

COMMENTS—The sample is sparse, consisting of one sporocarp and semi-permanent slide. The sporocarps of *Physarum hongkongense* are sessile, strongly compressed laterally, constricted at the base, and with a double peridium, the outer layer calcareous, crustose, bright yellow at the outer surface. *Physarum bogoriense* Racib. is a related species that differs from *P. hongkongense* by sporocarps that are not flattened laterally and the apical dehiscence in triangular lobes (Chung & Tzean 1998). There are no remarkable anatomical differences between the two species (spore measures and capillitial shape and colour), so the differences are only macroscopical (Moreno & al. 2017b).

Physarum leucophaeum Fr., Symb. Gasteromyc.: 24. 1818.

FIG. 11b

SPECIMENS EXAMINED: SPAIN, BADAJOZ, Azuaga, Aldea de Cardenchosa, on twigs of *Quercus obtusifolia*, 29-XII-1991, leg. J.R. García (AH 16245); Arroyo Argallón, on non-determined leaves, leg. J.R. García (AH 16259, with *Physarum album* and *Didymium*



FIG. 11. a. Sporocarps of *Physarum album* (AH 16259). b. Sporocarps of *Physarum leucophaeum* (AH 16259). c. Sporocarps with white pseudocolumella of *Physarum robustum* (AH 16407). Scale bars: a–c = 0.5 mm.

nigripes); on leaves of *Quercus obtusifolia*, 3-II-1991, leg. J.R. García (AH 16305); Calera de León, Cortijo La Cabra de Abajo, on wood of *Quercus obtusifolia*, 10-XII-1994, leg. J.R. García (AH 18430); Granja de Torrehermosa, Finca El Revuelo, on wood of *Pinus* sp. and leaves of *Nerium oleander*, 20-XII-1991, leg. J.R. García (AH 16251); Finca El Río, Zújar river, on twigs of *Quercus obtusifolia* and debris of *Juncus* sp., 24-VI-1992, leg. J.R. García (AH 16175, with *Didymium nigripes*); Arroyo Quejigo, on debris of *Populus nigra*, 12-XI-1993, leg. J.R. García (AH 48706, with *Didymium squamulosum*); Mérida, Cornalvo Roman water reservoir, on wood of *Quercus obtusifolia*, 20-XI-1993, leg. J.R. García (AH 16399); on wood of *Quercus suber*, 20-XI-1993, leg. J.R. García (AH 16418); Monterrubio de la Serena, Calabar mountain pass, on wood of *Quercus suber*, 13-XI-1993, leg. J.R. García (AH 48713); CÓRDOBA, Fuente Obejuna, Finca Las Canalejas, on wood of *Nerium oleander*, 7-I-1993, leg. J.R. García (AH 16145); Cortijo El Río, *Quercus obtusifolia*, 11-XII-1993, leg. J.R. García (AH 16426).

COMMENTS—Sample AH 16305 is interesting because it shows the sessile form of this species.

Physarum pusillum (Berk. & M.A. Curtis) G. Lister,
Monogr. Mycetozoa, ed. 2: 64. 1911.

SPECIMENS EXAMINED: SPAIN, BADAJOZ, Azuaga, Arroyo Argallón, on leaves of *Nerium oleander*, 3-X-1989, leg. J.R. García (AH 16728); Granja de Torrehermosa, Arroyo Parralejo, 480 m, on grasses, 19-X-1997, leg. J.R. García (AH 48735); Finca El Río, on grasses and debris of *Juncus* sp., 24-VI-1992, leg. J.R. García (AH 16249); close to the river, on riverside grasses, 15-XI-1994, leg. J.R. García (AH 48709); CÓRDOBA, Fuente Obejuna, Cortijo El Río, on grasses and debris of *Eucalyptus* sp., 1-XI-1997, leg. J.R. García (AH 46531, with *Didymium megalosporum* and *D. nigripes*).

COMMENTS—There are umbilicate sporocarps in samples AH 48709, AH 16249, and AH 48735. This form of *Physarum pusillum* was known and accepted by Lister (1925) and also by Poulain & al. (2011). In any case, *P. pusillum* has been compared with *P. xylophilum* Shuang L. Chen & Yu Li, a very similar species

described by Chen & Li (1998) that differs in its spore size and ornamentation. *Physarum pusillum* has spores 9–10 µm diam. and warted ornamentation with groups of warts, whereas in *P. xylophilum* the spores are 11–13 µm diam. and nearly smooth.

Physarum robustum (Lister) Nann.-Bremek.,

Proc. Kon. Ned. Akad. Wetensch., C 87(1): 91. 1984.

FIG. 11c

This species is characterised by its dark stalk and its white pseudocolumella from which the capillitium radiates. This is perfectly visible in the sample AH 20694.

SPECIMENS EXAMINED: SPAIN, BADAJOZ, **Peraleda del Zaucejo**, El Alcorcón meadow, on wood of *Eucalyptus* sp., 11-XII-1993, leg. J.R. García (AH 16407); CÁCERES, **Hoyos**, road to Valverde del Fresno, km 4, on leaves of *Castanea sativa*, 25-XI-1995, leg. J.R. García (AH 20694).

Physarum spectabile Nann.-Bremek., Lado & G. Moreno,

Proc. Kon. Ned. Akad. Wetensch., C 76(5): 484. 1973.

SPECIMENS EXAMINED: SPAIN, BADAJOZ, **Azuaga**, Fuente «La Jacoba», on cladodes of *Opuntia ficus-indica*, 29-I-1992, leg. J.R. García (AH 16130); 6-III-1992, leg. J.R. García (AH 16159); 10-X-1992, leg. J.R. García (AH 16133; AH 16151; AH 16171); 13-XI-1992, leg. J.R. García (AH 16340); 23-X-1993, leg. J.R. García (AH 16302); 16-I-1994, leg. J.R. García (AH 48664, with *Leocarpus fragilis*); 23-X-1997, leg. J.R. García (AH 48733, with *Badhamia gracilis*; AH 48752).

COMMENTS—Sporocarps of *Physarum spectabile* are greyish and often laterally compressed. Capillitium formed by small, globose, white nodes. Spores polyhedral.

Reticularia jurana Meyl., Bull. Soc. Vaud. Sci. Nat. 44: 297. 1908.

SPECIMEN EXAMINED: SPAIN, CÁCERES, **Guadalupe**, on trunk of *Eucalyptus globulus*, 7-III-1997, leg. G. Moreno & H. Kreisel (AH 48743).

Reticularia splendens Morgan, J. Cincinnati Soc. Nat. Hist. 15: 137. 1893.

SPECIMEN EXAMINED: SPAIN, BADAJOZ, **Azuaga**, Finca La Sierra, on stump of *Eucalyptus* sp., 1-XII-1989, leg. J.R. García (AH 48682).

Stemonitis axifera (Bull.) T. Macbr., N. Amer. Slime-Moulds: 120. 1899.

SPECIMENS EXAMINED: SPAIN, BADAJOZ, **Azuaga**, road to Cardenchosa, Benibezar river, on woody debris, 20-III-1987, leg. J.R. García (AH 16193); CÁRDABA, **Fuente Obejuna**, Cortijo El Río, 500 m, on wood of *Salix fragilis*, 22-IV-1996, leg. J.R. García (AH 16735); 1-XII-1997, leg. J.R. García (AH 46475).

Stemonitis flavogenita E. Jahn,

Verh. Bot. Vereins Prov. Brandenburg 45: 165. 1904.

Sporocarps violaceous-brown. Stalk and columella dark and shining, with the columellar apex ending in a funnel-shaped structure. Capillitium spiny and forming an external net with meshes of different diameters; main branches perpendicular to the columella. Spores 6.5–7.5 µm diam., globose to subglobose, pale violaceous-brown, verrucose.

SPECIMENS EXAMINED: SPAIN, CÁCERES, Jaraíz de la Vera, *Quercus pyrenaica*, 27-VIII-2001, leg. F. Prieto (AH 48745); CIUDAD REAL, Parque Nacional de Cabañeros, *Quercus pyrenaica*, 18-X-2001, leg. G. Moreno (AH 48750).

Stemonitis fusca Roth, Bot. Mag. (Römer & Usteri) 1(2): 26. 1787.

SPECIMENS EXAMINED: SPAIN, BADAJOZ, Azuaga, Arroyo Argallón, 540 m, on wood of *Nerium oleander* and twigs of *Rubus* sp., 19-X-1997, leg. J.R. García (AH 46530, with *Craterium leucocephalum*); Granja de Torrehermosa, Arroyo Quejigo, on wood of *Populus alba*, 19-XI-1994, leg. J.R. García (AH 16168); 24-I-1995, leg. J.R. García (AH 20691).

Stemonitis splendens Rostaf., Sluzowkce Monogr.: 195. 1874.

SPECIMENS EXAMINED: SPAIN, BADAJOZ, Azuaga, Arroyo El Cañaveral, on wood of *Nerium oleander*, 20-I-1995, leg. J.R. García (AH 48689); 24-I-1995, leg. J.R. García (AH 48693); CÁCERES, Hoyos, road to Valverde del Fresno, km 4, on wood of *Castanea sativa*, 25-XI-1995, leg. J.R. García (AH 20700).

Stemonitopsis typhina (F.H. Wigg.) Nann.-Bremek.,

Nederlandse Myxomyceten: 209. 1975 ["1974"].

SPECIMENS EXAMINED: SPAIN, BADAJOZ, Azuaga, Arroyo Argallón, on non-determined wood, 31-V-1993, leg. J.R. García (AH 16293, with *Comatricha* sp.); Mérida, Cornalvo Roman water reservoir, on wood of *Quercus suber*, 20-XI-1993, leg. J.R. García (AH 48671).

Trichia affinis de Bary, Jahrb. Nassauischen Vereins Naturk. 23–24: 336. 1870.

SPECIMEN EXAMINED: SPAIN, BADAJOZ, Azuaga, Arroyo Argallón, on wood of *Nerium oleander*, 7-VI-1991, leg. J.R. García (AH 16372, with *Arcyria cinerea*).

Trichia contorta var. *karstenii* (Rostaf.) Ing,

Trans. Brit. Mycol. Soc. 48: 647. 1965.

FIG. 12

SPECIMEN EXAMINED: SPAIN, BADAJOZ, Azuaga, Arroyo Argallón, on wood of *Nerium oleander*, 20-XII-1991, leg. J.R. García (AH 16480).

COMMENTS—This variety is represented by deep reddish brown sessile sporocarps to short plasmodiocarps, capillitial tubes with swellings and ornamented with tight spiral bands (Nannenga-Bremekamp, 1991).

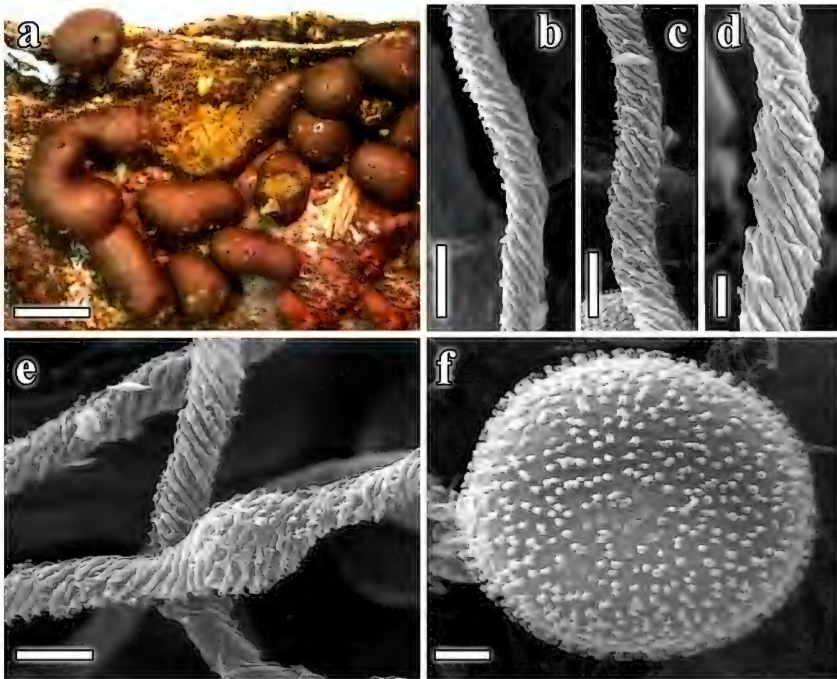


FIG. 12. *Trichia contorta* var. *karstenii* (AH 16480). a. Sporocarps. b-d. Detail of the capillitium (SEM). e. Swellings of capillitium (SEM). f. Detail of the spore ornamentation (SEM). Scale bars: a = 1 mm, b, c, e = 5 μ m, d, f = 2 μ m.

Trichia crateriformis G.W. Martin, Mycologia 55: 131. 1963.

FIG. 13

= *T. fallax* var. *olivacea* Meyl., Bull. Soc. Vaud. Sci. Nat. 44: 300. 1908.

= *T. decipiens* var. *olivacea* (Meyl.) Meyl., Bull. Soc. Vaud. Sci. Nat. 55: 244. 1924.

= *T. decipiens* f. *olivacea* (Meyl.) Y. Yamam., Myxomycete Biota Japan: 237. 1998.

Sporocarps stalked. Sporotheca greenish olive with dehiscence well marked by means of a circular area. Capillitium formed by pale yellow elaters (7–8 μ m diam.), ornamented with 3–4 broad and smooth long-tipped spirals. The capillitial elaters under SEM are smooth and marked with tightly-packed spirals. Spores globose to subglobose, 11–12.5 μ m diam., pale yellow by transmitted light, spinulose to crested ornamentation evenly distributed. Under SEM the spore ornamentation is cristate and formed by broad-based ridges that join to form short crests with irregular and sinuous morphology. Sometimes, they join towards the apex to assume a stellate or reticulate appearance.

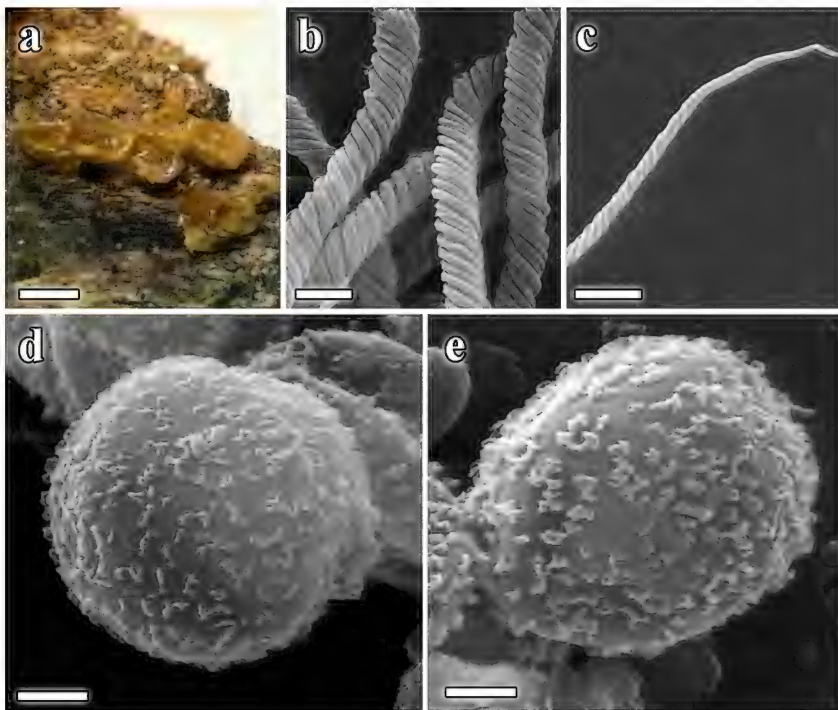


FIG. 13. *Trichia crateriformis* (AH 48744). a. Sporocarps. b. Detail of the capillitium (SEM). c. Detail of one elater of the capillitium (SEM). d-e. Detail of spore ornamentation (SEM). Scale bars: a = 1 mm, b-c = 5 μ m, d-e = 2 μ m.

SPECIMEN EXAMINED: SPAIN, BADAJOZ, Azuaga, Arroyo Argallón, on wood of *Nerium oleander*, 9-X-1992, leg. J.R. García (AH 48744, with *Arcyria cinerea*).

COMMENTS—At specific rank, *Trichia crateriformis* has priority over the three synonymous infraspecific names and must be used for this common species in the Mediterranean region. Moreno & Castillo (2013) studied the type specimens of these taxa.

Trichia varia (Pers. ex J.F. Gmel.) Pers., Neues Mag. Bot. 1: 90. 1794.

SPECIMENS EXAMINED: SPAIN, BADAJOZ, Azuaga, Arroyo Argallón, 540 m, on wood of *Populus nigra*, 6-XII-1995, leg. J.R. García (AH 20683, with *Ceratiomyxa fruticulosa*); Granja de Torrehermosa, Arroyo Quejigo, 540 m, on wood of *Populus alba*, 24-XII-1997, leg. J.R. García (AH 46474).

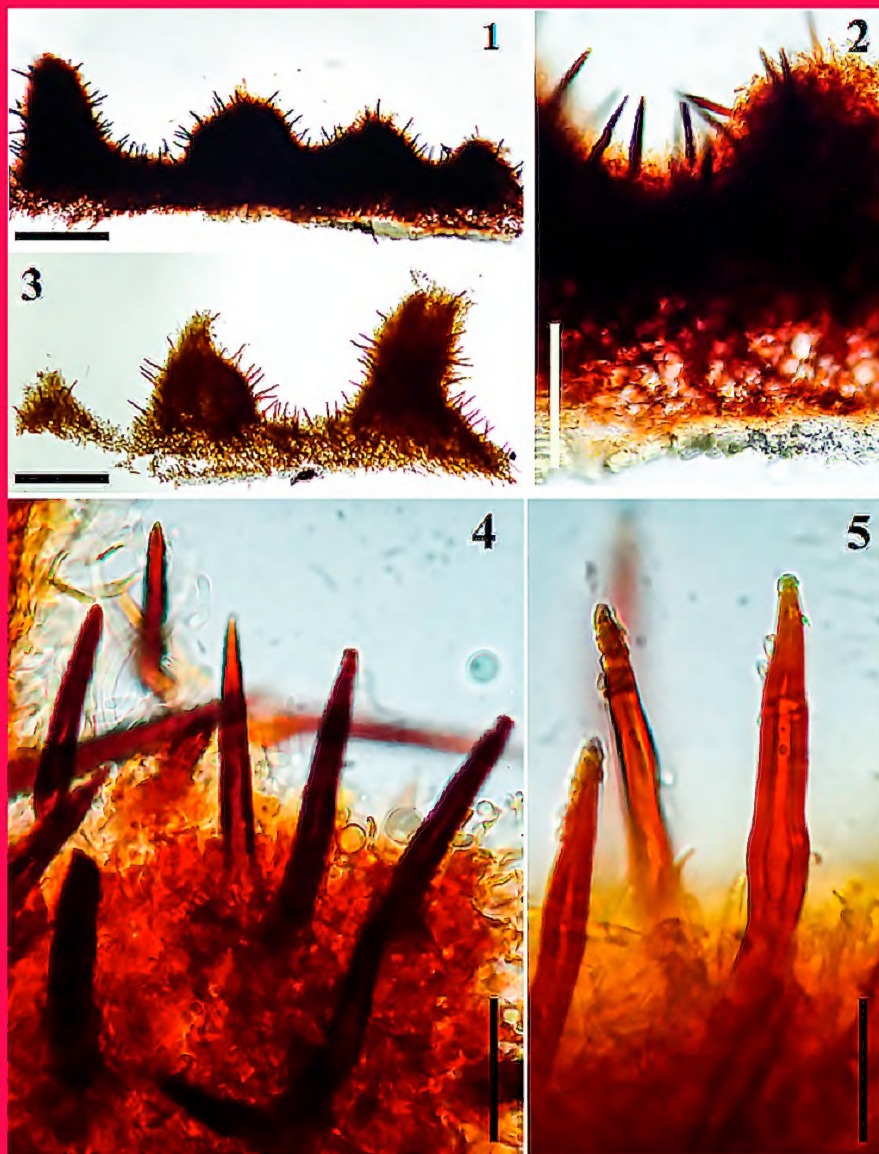
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(Contreras-Pacheco — PLATE 6, p. 508)